RESEARCH AND DEVELOPMENT ASSOCIATES FOOD AND
INSTITUTE, INC.
RATION ITEM STABILITY

By [illegible] Food & Container Institute

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1. Proteins
2. non enzymatic browning
3. browning reactions
4. ——— chemistry
5. color formation
6. Potatoes ⑨ reducing sugars
7. dried fruits
8. vegetables

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Contributions of

BROWNING RESEARCH

to

RATION ITEM STABILITY

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QUARTERMASTER FOOD AND CONTAINER INSTITUTE

SURVEYS OF PROGRESS ON MILITARY SUBSISTENCE PROBLEMS

SERIES I. FOOD STABILITY

1. Contributions of Browning Research to Ration Item Stability

A CONFERENCE ON THE STATUS OF BROWNING REACTION RESEARCH AND A REVIEW OF ITS CONTRIBUTIONS TO STABILIZED PACKAGED RATIONS HELD 1 FEBRUARY 1952 AT THE QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES (CHICAGO, ILLINOIS) RESEARCH AND DEVELOPMENT DIVISION, OFFICE OF THE QUARTERMASTER GENERAL.

Edited by Jack H. Mitchell, Jr., Stability Division, and Martin S. Peterson, Scientific Publications and Reports Office, Quartermaster Food and Container Institute for the Armed Forces

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FOREWORD

On behalf of the Research and Development Associates, Food and Container Institute, Inc., I am responding to an invitation to prepare a brief foreword for the papers and discussions which comprise this brochure. Perhaps the two questions of interest to readers about to undertake a study of the contributions of the research chemists who convened at the Institute on 1 February 1952 are: (1) What were the objectives of the conference? (2) Why are the Associates underwriting its publication?

As to the objectives of the conference, those immediately apparent were: To report the status of Quartermaster Corps contract research on browning; to evaluate research progress; and to assess the value of the different approaches selected by the various investigators in attempting to understand the mechanisms and control of browning. A further objective was to consider the present status of product applications—both those now in practice and those in prospect. Incidental to these objectives was an appraisal of research resources, not only as to funds but also as to talent—in short, the availability of research personnel for investigations of this type. It appears that the supply of food scientists does not balance the demand, and the situation will not soon be relieved.

Why do the Associates underwrite certain publications of the Quartermaster Food and Container Institute? It is of great importance to the industries to know what the supply problems of the Armed Forces are, what they are doing to solve or alleviate them, and what the food industry can do to accelerate their solution. The same information is also of great interest to the Military Establishment at large, to technical people, and to the general public. It bears on national defense.

Browning is one of the several major problems of concern in food deterioration. It is no respecter of products. Processors and handlers of milk products, of eggs, of baked products, meat, fruits and vegetables—all these and more—have long been troubled by the losses directly ascribed to the browning reaction. The complexity of the problem makes any advance—great or small—of significance. Through this brochure it becomes possible to convey to all who are technically concerned with the processing and storage of food an impression of the values already realized and those hoped for under the Institute's coordinated program on browning.

A. L. Elder

President, Research and Development Associates, Food and Container Institute, Inc.

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THE NON-ENZYMATIC BROWNING CONFERENCE

Quartermaster Food and Container Institute for the Armed Forces
February 1, 1952

Jack H. Mitchell, Jr., presiding

I. Opening Session

MITCHELL:

Of the fourteen contracts to study non-enzymatic browning made between the Quartermaster Corps and outside agencies since 1946, four are currently in effect. This meeting was called in order to appraise progress on these contracts and to exchange information and ideas pertinent to future lines of research. I take great pleasure in introducing at this time Dr. Tressler, Scientific Director, Quartermaster Food and Container Institute, who will outline the purpose and general subject matter of the conference.

TRESSLER:

As Dr. Mitchell has pointed out, this is primarily a conference for the purpose of reviewing the work currently being carried out on the so-called browning reaction under contract with this Institute. Great strides in the improvement of various dehydrated products are being made, and I wish to emphasize at the outset that a very considerable amount of the improvement has been made possible because of our growing knowledge of the browning reaction. For example, the dehydrated egg today as produced under contract for the Quartermaster is far different from that produced at the beginning of World War II. At that time the product would not stand storage. Today it is to a large extent stabilized. This fact is worth a moment of background comment. It was learned that the browning reaction goes on less readily at a relatively low pH. From that finding it was reasoned that if the acidity were increased (the pH reduced) the reaction could be slowed down materially. Further, it was demonstrated that the reaction proceeds much more slowly at low moisture levels. Later work has shown that if the reducing sugar is largely or wholly eliminated the reaction is reduced to the minimum.

Based on these findings, several successful processes of egg dehydration have been developed. In one, increased acidity is obtained by the addition of hydrochloric acid. The egg is then flash-pasteurized, thereby partially inactivating the enzyme as well as reducing the *Salmonella* count. Then the egg is dehydrated to a low moisture percentage and sodium bicarbonate is added. When the egg is reconstituted in water it tastes like fresh egg to which salt has been added. In another process the reducing sugar is fermented and thus eliminated. In still another process an enzyme is added to oxidize and thereby remove the reducing sugars. Any one of these processes produces an egg of high quality. Without our knowledge of the browning reaction we certainly could not have achieved these results.

To some extent our success in dehydrating potatoes has also been due to our knowledge of the browning reaction. It is probable, of course, that we have not made all the use of the knowledge of the browning reaction that we should in this case. For example, the specification for dehydrated potatoes still allows 7% moisture. That is too high. It should be down to 5% or even 4%. We know that dehydrated potato granules can be reduced to a 4% moisture level if the right processes are used. Further, dehydrated potato contains some reducing sugars. Since it is established that the lower the reducing sugars, the longer dehydrated potato will keep in storage, we should require the reducing sugar level to be very low and to indicate exactly the maximum amount permissible. This is not an unrealistic request since we know that much of the reducing sugar in the potato, left there at the initial stage of processing, can be eliminated by special treatment. Dr. Pyke will tell us what his group has done on that score, and I think you will agree that we have come a long way toward the solution of the dehydrated potato problem. In the future we should have a dehydrated potato satisfactory in all respects.

Despite the accomplishments just described, I feel that we should be progressing faster than we have. It would be beneficial to have a coordinated plan whereby the knowledge obtained from contract research could be funneled to our technologists for study and for early application to the improvement of dehydrated products. This may mean some changes in approach. To elucidate this point—I made a trip around the country and talked to our contract workers in this field. I pointed out during my visits that the work being carried on is mainly on products in aqueous solutions and relatively dilute solutions at that. I have felt for a long time that conclusions drawn from model systems using dilute solutions do not reveal the real changes which occur in natural products. Moreover, I observed that much of the work is concerned with simple browning. Now color may be an index value in estimating the extent of the reaction, but so far as the food technologist is concerned, color changes are relatively unimportant compared to flavor changes. If there is a color change, as in dehydrated onions, the flavor may not be ruined. But if the flavor of a product *is changed*, then the product may be rendered substantially worthless. The cause of flavor change, then, becomes the quarry we are after.

I hope that you will be very frank in your discussions today. If you feel that we are not going to get anywhere in the immediate future, say in the next year or two, I wish you would tell us so. If you can guide us so that we can eliminate work that is of only marginal interest to the technologists. I hope you will not hesitate to do so. This is an occasion for very frank criticism and even self-criticism. Self-criticism, I believe, is sound scientific policy. In view of our limited funds, it is certainly a necessary policy.

When in England during the last part of November and early December, I enjoyed a visit with a noted chemist who is studying the changes which occur in the products when they are in the dry condition. By varying the amount of humidity in the atmosphere he has found out certain fundamentals of this so-called browning reaction. The man I refer to is with us

today, and it is now my privilege and honor to introduce Dr. Colin Lea of the Low Temperature Research Station of Cambridge, England. He will tell us about the work that he has been carrying out and is currently carrying out on browning as it occurs in products of extreme low moisture content. Dr. Colin Lea:

Review of Work at the Low Temperature Research Station on the Reaction Between Proteins and Reducing Sugars in the "Dry" State

LEA:

Before proceeding to review briefly the work which my small group at the Low Temperature Research Station in Cambridge has been carrying out during the last two or three years on certain aspects of the non-enzymic browning reaction, I would like to sketch in a little of the background against which this work must be considered.

In the first place one cannot stress too often the fact that, while the chemical reactions grouped loosely together under the general term "browning reaction" do have something—and perhaps quite a lot—in common, their mechanism must vary widely with the chemical composition of the different foods in which they occur, and probably also in some degree with the conditions of processing and storage.

You will need no reminder from me of the work of the University of California group on apricot concentrate, wherein browning was found to be producible by the interaction of the nitrogenous constituents and the sugars, from the nitrogenous constituents and the organic acids, from the sugars and the organic acids, or from the organic acids fraction alone. In citrus fruit juices, too, ascorbic acid seems to play a major part in browning, though obviously it could not be so important a factor in the browning of, for example, dried egg or milk powder.

My first point, then, is to stress that our investigations have been limited to one type of browning reaction; namely, that occurring in systems rich in amino-N-containing material (usually protein) and low in ascorbic and similar acids, and not too far removed in pH from neutrality.

Also, we have investigated this reaction in the *solid* state at moderate storage temperatures, and have preferred, on the whole, to work with *proteins* rather than with amino acids.

Furthermore, from lack of manpower rather than from deliberate choice, we have confined our attention mainly to the *earlier* stages of the interaction between proteins and sugars, and have largely neglected the chemistry of the processes by which the dark substances themselves are produced. In these respects our work and the work of our American colleagues have been quite largely complementary.

FOOD SYSTEMS

Our interest in the browning reaction in high protein foods commenced with work during the war on dried eggs. Later we studied the same reaction in non-fat dry milk and were able to demonstrate (Figure 1) a stoichi-

ometric relationship between the loss of free amino groups and the binding of lactose by the protein *(1)*.

This protein-sugar interaction in milk powder was, of course, mainly obvious at high moisture contents, where a marked loss in nutritive value also occurred *(1)*. At low moisture contents some protein-sugar interaction

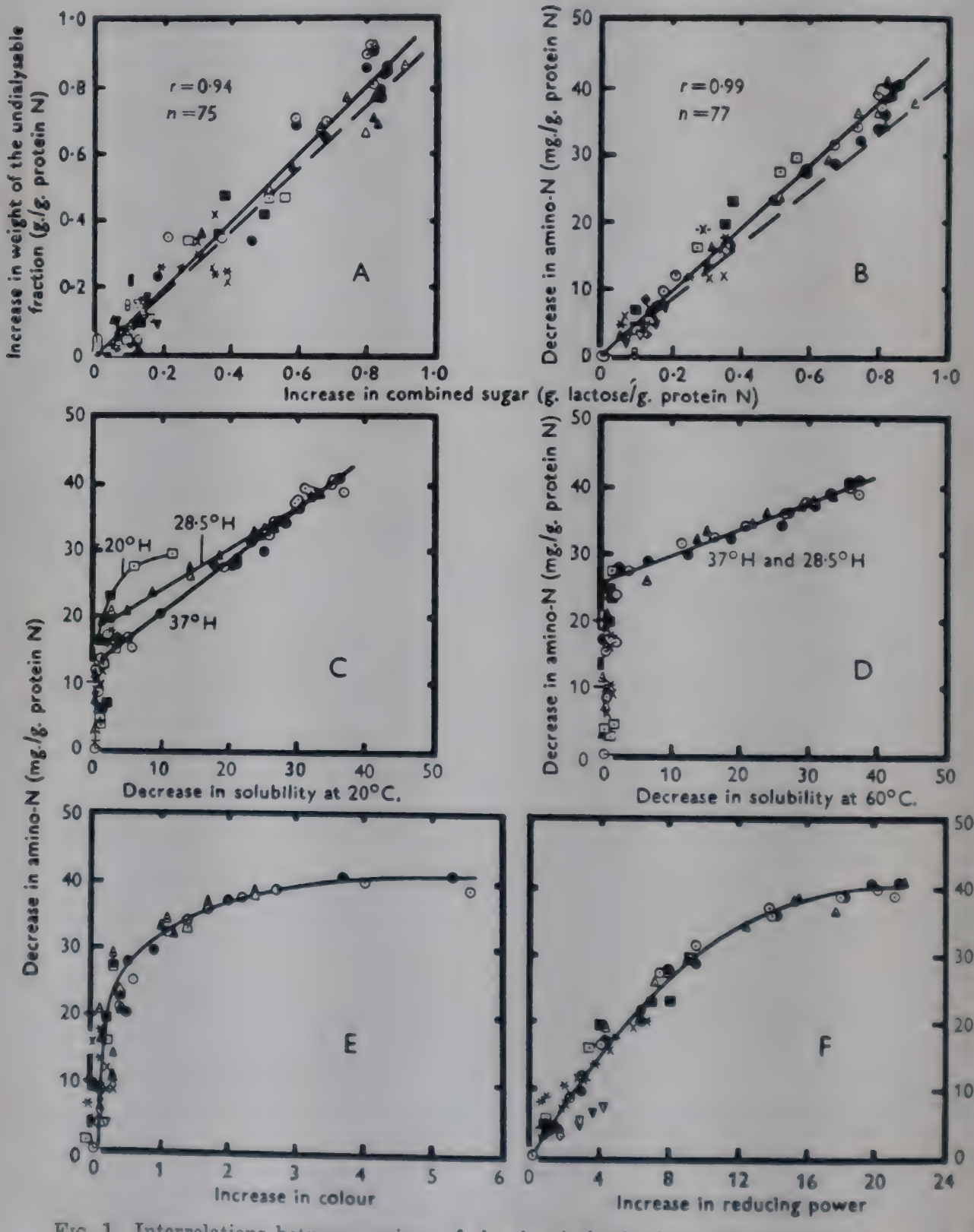

FIG. 1. Interrelations between various of the chemical criteria used in a study of the effect of storage on milk powder. Legend: samples of high moisture content (7%) stored at 37°C. = ○, 28.5°C. = △, 20°C. = □; samples of medium moisture content (5%) stored at 37°C. = X, 28.5°C. = ◇, 20°C. = ⅄; samples of low moisture content (3%) stored at 37°C. = ▽, 28.5°C. = ⊡, 20°C. = ⊡. Storage in nitrogen is indicated wherever the above symbols are shown in black.

was still detectable but was now very slow. Moreover, at low moisture contents oxidative changes, probably in the small amount of lipid still present in the non-fat milk solids, produced an adverse effect on flavor after long storage, which was greatly reduced by packing in inert gas. It was interesting to find that non-fat dry milk, which is usually considered a fairly stable commodity, kept much better in an atmosphere of nitrogen than in air whether the moisture content was high or low. At high moisture contents it is true that neither the development of brown color (which only occurred with extreme deterioration) nor the development of insolubility of the protein was affected by the atmosphere, but the production of CO_2 by the powder was ten times greater in air than in nitrogen, and the "off" flavor developed in air was different in quality and more objectionable than that developed in nitrogen *(1)*.

But we soon felt that the amount of progress that could be made in elucidating the complicated series of reactions involved in browning by experiments on a complex food material was very limited, and we turned perforce to simpler systems. First we used dialysed milk protein with sugars *(2)*, then individual proteins with sugars, and finally individual protein groups with sugars.

PROTEIN-SUGAR SYSTEMS

From experiments with freeze-dried casein-glucose mixtures, we were able to show that the reaction between the protein amino groups and glucose is slow in aqueous solution and in the dry solid but proceeds at a maximum

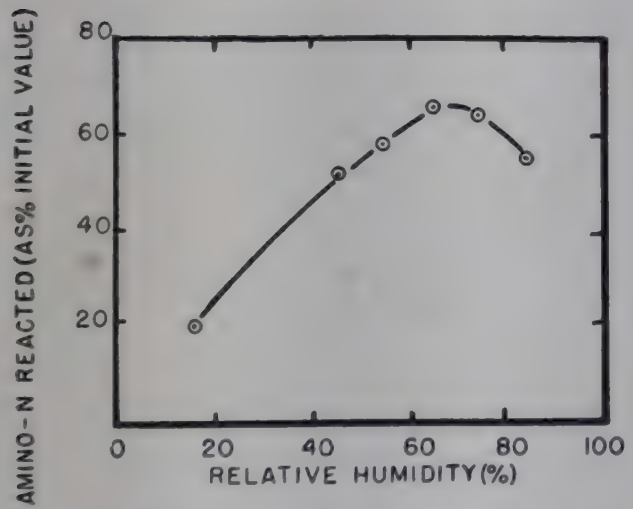

FIG. 2. Influence of moisture levels on percentage of amino-N combined with glucose in freeze-dried insulin-glucose mixture.

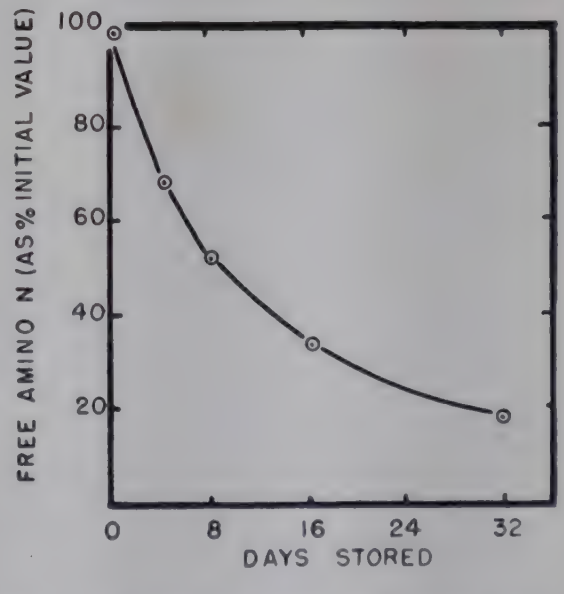

FIG. 3. Showing rate at which free amino-N combines with glucose during storage of freeze-dried insulin-glucose mixtures.

rate in the moist solid at a moisture content corresponding to a relative humidity of 65-70%* *(3)*. This finding has recently been confirmed for insulin, and with insulin, moreover, it has been possible to follow separately the reaction with glucose of the ϵ-amino groups of the lysine side chains

* Browning, however, continued to increase at least up to 85% R.H.

and of the α-amino groups of the glycine and phenylalanine residues at the ends of the peptide chains *(4)*. (See Figures 2-4.)

With dried human blood plasma—which we investigated—because in Europe it has been the practice to add extra glucose to the plasma before freeze-drying it—the same kind of reaction can go on during storage *(5)*.

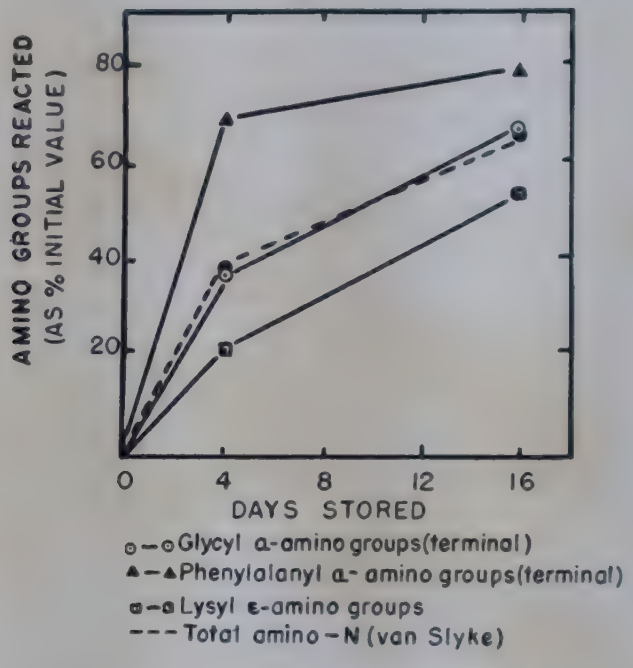

FIG. 4. Reaction of three amino groups and glucose in terms of days stored.

Here also there is a relative humidity at which the reaction proceeds at a maximum rate—somewhat lower in this complex system than with the pure proteins. In all of these protein systems, however, the optimum conditions for the reaction seem to correspond with the point at which the vapor pressure isotherm swings sharply upwards, presumably following the covering of the most hydrophylic sites of the protein with water.

The reaction between glucose and casein was shown to reach a maximum rate at a concentration of glucose which would cover with a monolayer the same area as the water known to be present *(6)*. It is interesting, therefore, to speculate that the amino group-sugar reaction proceeds most rapidly in a monolayer of water at the protein surface, covered in turn by a monolayer of glucose. It must be pointed out, however, that we have recently observed optimum R.H. values qualitatively similar but quantitatively different for the reaction of simple molecules such as α-N-acetyl-L-lysine, which can hardly be considered to possess a surface in the sense that a protein does, although the conception of the reaction as taking place in a film of water held by the polar groups is perhaps still feasible.

Other characteristics of the amino group-sugar reaction were found to be its high temperature coefficient ($Q_{10}^{15\ 25^{\circ}C.} = 5.4$)[3], and the increasing rate of reaction with pH *(3)*. Browning and insolubility developed only after a lag period which was sufficiently marked to permit the separation of a

casein-glucose complex which had lost 70% of the original free amino groups and contained approximately 7% of firmly bound glucose, but which was still practically uncolored and fully soluble *(7)* (Figure 5). The effects of pH and moisture content on browning are shown in Table 1.

TABLE 1

The Effect of Activity of Water and of pH on the Development of Color in Casein-Glucose

% R.H. (at pH 6.3)	Lovibond Y + R units after days at 37°C.					
	2	4	8	16	32	64
20	....	....	....	....	....	0.2
40	....	....	....	0.0	0.1	0.6
55	....	....	....	0.1	0.8	1.5
70	....	....	0.0	0.8	1.9	2.7
85	....	0.1	0.8	1.9	3.7	5.0
92½	....	0.1	0.8	1.9	3.3	....
96¼	....	0.1	0.8	2.1	....	....
pH (at 70% R.H.)						
3.0	....	....	0.1	0.2	0.5	1.1
4.6	....	....	0.2	0.4	1.0	1.9
6.3	....	....	0.1	0.9	1.8	2.6
7.0	....	....	0.3	1.1	2.0	2.9
8.0	....	0.0	0.7	1.6	2.4	3.4
9.0	0.1	0.4	1.2	2.2	3.0	3.8
10.0	0.2	0.8	1.7	2.6	3.4	4.4

When changes in the amino acid composition of the casein were investigated, it was found that combination of glucose with free amino groups (i.e., reaction with the lysine side chains) accounted for a large proportion of the bound glucose present in the still white and soluble product. Several

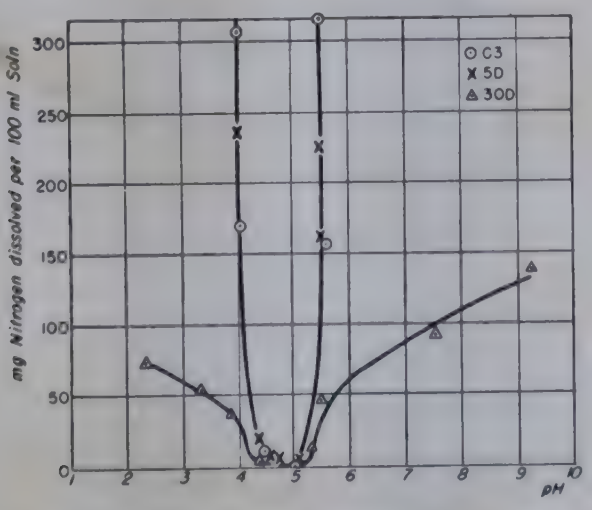

FIG. 5. Solubility of casein (C3); casein reacted with glucose at 37°C. and 70% R.H. for 5 days (5 D); and 30 days (30 D).

other amino acids including arginine and histidine, and probably tyrosine and methionine, had also reacted extensively in the badly deteriorated, brown and insoluble product *(7)*. Estimation of the extent of reaction of individual amino acid side chains in a protein-sugar complex is, however, a matter of some difficulty since accurate amino acid analyses are usually carried out after complete hydrolysis of the protein. This procedure seems to be satisfactory in the case of arginine and histidine, where estimations on the intact complex and on the HCl hydrolysate gave the same result, indicating that these amino acids are not regenerated from their compounds with sugar by the action of concentrated mineral acid. About two-thirds of the lysine which has reacted with sugar, however, seems to be regenerated on acid hydrolysis, although the lysine-sugar complex is probably resistant to enzymic hydrolysis in the gut. The probable losses of methionine and tyrosine were indicated by colorimetric methods applied to the intact or nearly intact protein-sugar complex. These admittedly inexact techniques indicated that both amino acids had reacted with glucose to a considerable extent in the badly deteriorated protein, although they were practically completely regenerated on hydrolysis of the protein with acid or alkali.

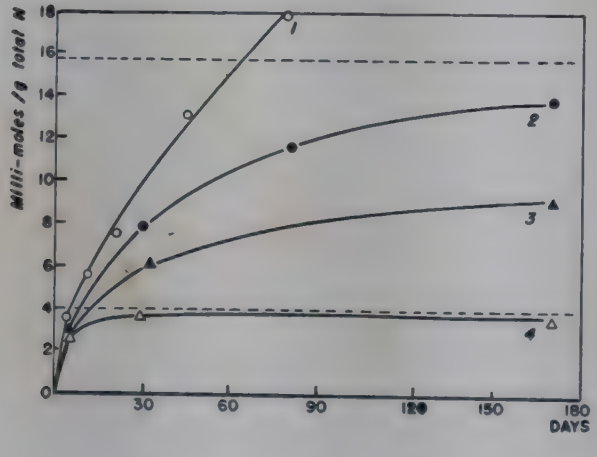

FIG. 6. Production of water during reaction of casein with glucose at 69% R.H. and 37°C. in nitrogen. Legend: 1 = water produced; 2 = glucose reacted; 3 = carbohydrate bound; 4 = amino groups reacted. Dotted lines = amount of amino-N and of glucose in mixture.

The increasing amounts of glucose reacting during prolonged storage, the proportion remaining bound to the protein, and the amount of water liberated during the reaction are shown in Figure 6. A marked deterioration in the biological value of the protein can be produced by as little as 5-30 days' storage at 37°C. (Table 2).

TABLE 2

Changes in the Nutritive Value of Casein Stored with Glucose Under Nitrogen at pH 6.3, 70% R.H. and 37°C.

Length of storage (days)	Biological value		True digestibility		Protein efficiency	
	Mean value for 12 rats	Standard error	Mean value for 12 rats	Standard error	Mean value for 12 rats	Standard error
0	77.9	± 1.38	99.5	± 0.28	2.42	± 0.300
5	61.6	± 1.11	96.6	± 0.67	1.95	± 0.103
30	38.7	± 1.00	90.6	± 1.25	0.32*	.....

* Two rats lost weight.

REACTION OF CASEIN WITH CARBOHYDRATES OTHER THAN GLUCOSE

A limited amount of work has also been carried out on the reaction of carbohydrates other than glucose with casein. The well-known decreasing rate of reaction in passing from the aldo-pentoses through the hexoses to the disaccharides has been confirmed for the dry system, and an exceptionally rapid rate of browning in relation to amino-N change observed with the uronic acids *(10)*. It was shown that 2-deoxygalactose, which was investigated as an example of a sugar modified at the apparently important 2 position, reacted with the amino groups of casein at only one-third of the rate of galactose, but the reaction mixture—rather unexpectedly—browned about 6 times as rapidly.

D-Glucosamine was also investigated as an example of an aldohexose in which the important 2 position is blocked, this time by an amino group. The results, unexpected and different from anything we have met previously, will be referred to again later.

MODIFIED PROTEIN SYSTEMS

We have now surveyed in a very superficial way the interaction between protein and reducing sugar in the "dry" state, and have shown that the most rapid reaction occurring is that between the protein amino groups and the potential aldehyde group of the sugar. Under suitable conditions this reaction can have proceeded extensively although the reaction mixture is still practically colorless and the protein fully soluble. This state of affairs obviously permits the possibility of a food appearing to be all right and yet having lost some of its biological value owing to reduced availability of its lysine.

We have also shown that, following upon the loss of amino groups other protein groups such as arginine and histidine, and possibly methionine and tyrosine become heavily involved. Since this latter phase of the reaction often corresponds with extensive browning the question naturally arises as to how far the objectionable consequences of the protein-sugar interaction are due to the reaction of the amino groups of the protein, and in how far to the reaction of these other protein groups.

In a first attempt to solve this problem, we prepared a casein-glucose complex in which glucose had combined with about 70% of the free amino groups of the casein, but with only minor proportions of the other reactive groups. When stored in the absence of free glucose this complex was found to brown rapidly at a rate which indicated that decomposition of carbohydrate attached to the protein amino groups could account for a large part, although probably not for all, of the darkening of a casein-glucose mixture at 37°C. *(8)*. The reducing power characteristic of the amino-glucose complex also decreased, and insolubility developed, the change in each case being more rapid at 85% than at 69% R.H. Only a small proportion of the carbohydrate became dialysable and practically no water was produced. No further loss of free amino groups occurred and arginine disappeared only very slowly.

Apparently, therefore, cross linking between carbohydrate already attached to amino groups and other amino or guanidine groups is not quanti-

tatively an important factor in the "dry" casein-glucose system. It should be pointed out, however, that even a very few cross linkages, if formed between molecules, could cause a very large increase in molecular weight with consequent changes in physical properties.

In a second attack on the problem of the part played by the protein amino groups we prepared an acetylated casein in which about 95% of the free amino groups had been blocked by acetylation. This acetylated casein, when stored with glucose, browned only very slowly at 37°C., thus confirming the importance of free amino groups in the browning process *(8)*. Arginine (and apparently other non-lysine side chains) in the acetylated protein still reacted with glucose, although more slowly than in casein, and the capacity to reduce ferricyanide at pH 5 increased. At 60°C. all three changes were accelerated about 20 times, very pronounced darkening occurred, much glucose combined with the protein and all the arginine groups were rapidly destroyed, notwithstanding the absence of free amino groups from the system.

The presence of free amino groups in a protein is therefore not essential for interaction between a protein and a reducing sugar, but deterioration certainly proceeds much more rapidly or at a lower temperature when amino groups are present.

MODEL SYSTEMS

In a third method of attack on this same problem the mechanism of the reaction between the isolated terminal amino group of lysine and glucose has been studied in somewhat greater detail by the use of two synthetic substances, namely α-N-acetyl-L-lysine and polylysine. Again freeze-dried mixtures of the amino compound with glucose, with an initial pH on resolution of 6.5, were used *(12)*.

The results obtained were strikingly similar in many respects to those already observed with the protein-sugar reaction, and again confirmed the importance of the part played by the lysine ϵ-amino groups in this reaction.

At high humidities a typically complex Maillard reaction was observed but distinct phases in the reaction could be recognized by controlling the R.H. of storage.

At 20% relative humidity (R.H.) the reaction was relatively simple. Amino groups and glucose disappeared in equimolecular amounts, no change in pH occurred and the color development was negligible; the samples were still white after six days and only pale cream after 24 days when well over half the amino groups had reacted. A small peak developed in the ultra-violet absorption spectrum at about 2960 Å.

Increasing humidities of storage caused an increased rate of loss of amino groups until a maximum was reached at 40% R.H., but greater complexity of the reaction was now also evident. In particular, after an initial time-lag, the peak in the ultra-violet region increased considerably, and shifted slightly to 2980Å, while a small amount of undifferentiated absorption extending well into the visible region gave rise to definite browning. A small but significant loss of glucose in excess of a one-to-one ratio, and a fall in pH also occurred.

At 60% R.H. the rate of loss of amino groups had fallen off considerably, but the development of absorption at all wave lengths, the fall in pH and the loss of excess glucose all reached a maximum. The absorption in the visible region (browning) was now relatively much greater than at 40% R.H., and a further shift of the peak to 3020 A had occurred.

Further increase of humidity produced no other well-marked changes in the character of the reaction. The absorption spectrum remained generally similar to that at 60% R.H. and the other effects, except possibly the pH drop, developed less rapidly as the humidity rose.

DEMONSTRATION OF INTERMEDIATE COMPOUNDS

After several days' reaction the pH had usually departed considerably from the original nearly neutral conditions and entered the acidic region where free furfurals are known to form. The search for intermediate products was therefore confined to the first six days of reaction or less, during which pH changes were small.

Descending paper chromatography in 80% aqueous propanol was used, and under these conditions unreacted glucose and acetyl lysine separated easily from the reaction products.

PRODUCTS AT 20% R.H.

At 20% R.H. the reaction product was found to run essentially as a single well-defined spot of RF value 0.1 which had about the expected nitrogen content for a simple 1:1 acetyl lysine-glucose combination. The spot showed a blue fluorescence on heating the paper and positive reactions on heating with ninhydrin and ammoniacal silver nitrate. It also reacted positively to the Elson and Morgan test for N-acetyl glucosamine, and reduced ferricyanide at pH 5. No glucose, however, could be recovered by hydrolysis with acid as would have been expected had the substance been a simple N-glycoside. In view of the possibility that an originally formed N-glycoside might have undergone the Amadori rearrangement to an isoglucosamine, reduction of spot A by palladium and hydrogen in ethanol was attempted, under conditions known to reduce aromatic isoglucosamines. The result, however, was indefinite, and the precise constitution of substance A remains undetermined.

At 40% and higher R.H.'s, a number of slower moving bands (BDEF in Figure 7) appeared. These substances were colorless but fluoresced under ultra-violet light, B—which appeared as a characteristic narrow band of R.F. 0.9 on the rear edge of Band A—in particular showing a brilliant purple fluorescence. A colorless non-fluorescent compound with an absorption peak at 2800 A was isolated from space C. G represents the brown products of the reaction, which were now beginning to appear, and which failed to move on the paper.

None of these substances has been identified owing to the small quantities separated. They could not be identified with any of the products of the reaction of hydroxymethylfurfural with acetyl lysine.

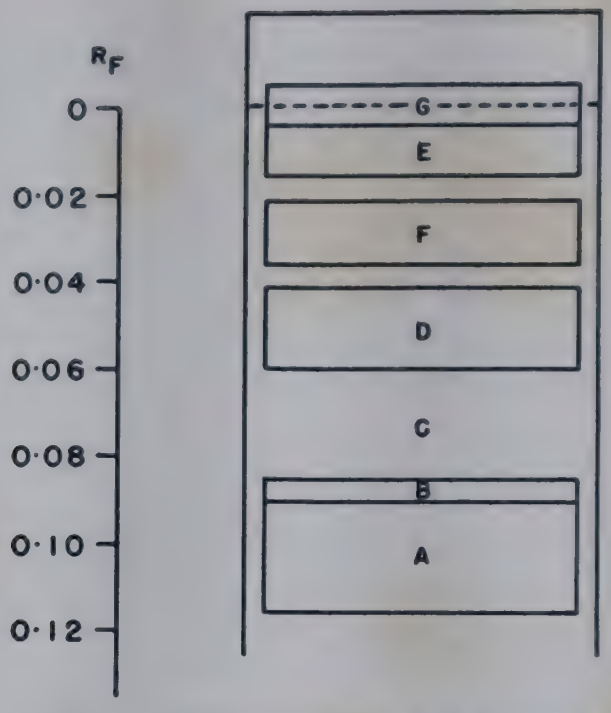

FIG. 7. Chromatogram of d-N-acetyl-lysine-glucose reaction product.

BREAKDOWN OF THE INTERMEDIATE COMPOUNDS

When the developed chromatograms were heated at 80°C. for a few hours, pronounced browning only appeared in region A, suggesting that this substance is one of the main intermediates in the browning reaction.

Small samples of substance A were therefore prepared by reaction of α-N-acetyl lysine with glucose for 6 days at 20% R.H., followed by paper chromatography of the reaction product and elution of band A from the paper. This material was adjusted to pH 6.5, freeze-dried and stored at 37°C. and various relative humidities.

Strong browning and a fall in pH were found to develop without any time lag and with a maximum rate at 60% R.H., thus providing a qualitative explanation of the behavior of the intact reaction mixtures. The degree of browning produced, however, was only about one-third of that expected, but if fractions C-F were added back again to fraction A, the mixture browned at the full rate. Apparently some component of the substances C-F, which does not brown itself, nevertheless increases the degree of browning produced by A.

REACTION OF POLYLYSINE WITH GLUCOSE

The results of the polylysine-glucose experiments were strikingly similar to those obtained with the α-N-acetyl lysine-glucose system. In this case molecules were too large for chromatographic separation of intermediate reaction products, but the system offered the advantage that insolubility developed similar to that observed with the true proteins. The colorless polylysine glucose complex formed at low humidities was freely soluble, but the dark brown product of extended reaction at higher humidities was insoluble. Before insolubility developed, a marked increase in mean molecular weight from the 11,000 of the original polymer to about 20,000 was detected.

Again the colorless intermediate complex prepared by reaction of polylysine with glucose at a low relative humidity was isolated, this time by removing excess glucose by dialysis, and stored at 40, 60, and 80% R.H. Again it browned with a maximum rate at about 60% R.H. and became insoluble.

It is perhaps of interest at this stage to review briefly the points of similarity between the three systems, casein, α-N-acetyl lysine, and polylysine. In all three:

(1) The rates of the primary reaction between free amino groups and glucose are similar.

(2) Browning develops only after a distinct time lag during which the primary reaction proceeds.

(3) There is a clearly defined optimum humidity for the reaction of amino groups and an appreciably higher optimum humidity for browning.

(4) Equimolecular losses of glucose and amino groups, indicating a stoichiometric reaction, are observed while the reaction mixtures are colorless.

(5) Browning is accompanied by a loss of glucose in excess of the 1:1 ratio and by a fall in pH.

(6) The amino group is still basic after reaction with glucose (shown by titration with the protein and by the absence of pH changes in the model systems under suitable conditions).

(7) Acid hydrolysis fails to regenerate glucose.

(8) Reducing power towards acid ferricyanide and a positive reaction to the Elson and Morgan test develop.

(9) Blue fluorescence appears at the higher humidities.

(10) Isolation and storage of the first-formed colorless complex produces browning and (except with α-N-acetyl-L-lysine) insolubility.

The absorption spectra observed with the model systems could not be demonstrated with casein owing to the strong absorption of the protein in this region, but the general correspondence listed strongly suggests that basically the same reaction is occurring in all three systems.

Most of these properties can be explained in terms of formation and degradation of a colorless intermediate compound, but whether or not this is the complete story I am not prepared to say. Further work with model systems along these lines—and the work I have outlined can only be considered a beginning—should lead to interesting results.

THE GLUCOSAMINE REACTION

In conclusion, it will, I think, be of interest to mention briefly a case of browning in these dry protein-carbohydrate mixtures which appears to be different in type from any we have so far discussed and which may perhaps give us a new lead into some aspects of the problem *(13)*.

As was brought out earlier, we attempted to use glucosamine (2-deoxy-2-aminoglucose) as a reducing sugar modified in the important 2 position, and one, moreover, which is known to be extremely resistant to acid hy-

drolysis. We hoped to be able to recover it undecomposed by acid hydrolysis of its complex with casein.

In fact, when we stored a freeze-dried mixture of glucosamine hydrochloride and casein, which had been adjusted to our usual pH of 6.3 and R.H. of 70%, a phenomenally rapid browning of the reaction mixture was found to occur, but the chemical data indicated that something entirely different from the normal protein amino group-sugar aldehyde group type of condensation was taking place. The free amino groups of the protein were found not to be involved at all—or at least to remain unaffected by the end of the reaction—while the amino and aldehyde groups of the glucosamine were both rapidly and simultaneously destroyed.

This glucosamine reaction is accompanied by a marked browning of the reaction mixture, and the free HCl released as the amino groups of the glucosamine are destroyed depresses the pH and renders the casein insoluble in the region of the isoelectric point (pH 4.6).

As with the amino-aldehyde reactions already investigated, the rate shows a striking dependence on the water content of the material, being very slow in the dry solid or in an aqueous solution and reaching a maximum at an intermediate water content corresponding in this case to a relative humidity of 80%.

In a search for the explanation of this behavior we took a series of solutions of glucosamine hydrochloride in water, adjusted their pH's to a series of values between 5.1 (which corresponds to 100% hydrochloride) and 9.2 (which corresponds to 100% free base), and freeze-dried them and stored at 37°C. and 70% R.H. An extremely rapid disappearance of glucosamine —as estimated by the Elson and Morgan Method—occurred, which stopped. however, as soon as all the free base present in the mixture had been used up. At pH 6.3 in aqueous solution only about 0.5% of the glucosamine is present as the free base, and the mixture is correspondingly stable.

Glucosamine is therefore destroyed on drying and storing with protein at a pH at which it would be expected to be stable, the protein in the semi-dry state apparently neutralizing the hydrochloric acid and leaving the free base to react in the water film on the protein surface.

N-ACETYL GLUCOSAMINE

A comparable phenomenon was observed when N-acetyl-D-glucosamine was dried and stored in the same way with casein *(13)*. After storage the reaction product was found to react in the Elson and Morgan method for N-acetyl-glucosamine *without* the preliminary treatment with alkali to give a color spectroscopically identical with that obtained in the normal method. This indicates that the isomerization and condensation to the oxazole ring, which is usually brought about by boiling with 0.5N Na_2CO_3, had proceeded on the surface of the "dry" protein.

In both of these reactions the surface of the protein appears to have provided a more alkaline environment than would be expected from the pH of the solution from which it was dried. Such factors must obviously be included in consideration of deterioration of the browning type in dried food-

stuffs, where degradation and caramelization of carbohydrate frequently occurs under conditions of pH at which the sugars alone would be stable.

It has not been possible within the compass of so brief a review to comment on the numerous excellent papers from other laboratories—virtually all American—which have appeared during the last few years. Some of these publications, and in particular those of Olcott and his collaborators at the Western Regional Laboratory, have employed approaches not very different from our own. It is only, however, by studying results from such varied methods of attack as we will hear about today, that really rapid progress in this immensely complicated but fascinating subject is likely to be achieved.

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II. Current Investigations

Work of the University of California Group on Sources of Non-Enzymatic Browning In Dried Fruits and Vegetables

MACKINNEY:

The Division of Food Technology, University of California, maintains an active interest in non-enzymatic browning of fruits and vegetables because it is a major cause for deterioration in products conservatively valued at seventy to one hundred million dollars a year.

Active participation is justified by the progress made elsewhere with certain commodities, notably dried eggs, dried milk and dehydrated potatoes, and is encouraged by the so-called "model system" studies which are fundamental to an understanding and control of the deterioration.

Dried apricots were chosen for two reasons:

1. They ordinarily darken more rapidly than other fruits, with greater loss in palatability.
2. Properly processed and prepared, they are highly acceptable and to military personnel subsisting on packaged rations they should have particular appeal.

Studies up to 1949 yielded basic and practical information on the relation of various harvesting, processing and storage factors to browning, specifically, the effect of the degree of maturity of the fruit when picked, various methods of control during sulfuring and drying, storage temperatures. storage in air vs. nitrogen, moisture content, etc.

As a result of these studies, the reason for many trade practices became apparent. The findings may be summarized as follows: Oxygen uptake by the dried fruit causes additional losses in sulfur dioxide content. The rate of uptake decreases as the moisture content is lowered. Thus, bin-stored fruit, with unlimited access to air, will darken less at 16-17% moisture than at 22-23%.

On the other hand, the view that a fruit with 1000-2000 p.p.m. of sulfur dioxide would not darken was shown to be untenable. This finding made it clear that the role of sulfur dioxide in retarding the browning of fruits was not limited to its reducing power. At least three roles can be postulated for sulfur dioxide with supporting evidence for each: (1) reducing action; (2) bleaching action; (3) an ability to form addition-compounds with substances that react to cause browning.

Among the internal factors studied, the role of the sugars has been examined most intensively. Removal of the sugars by fermentation or by ion-exchange techniques causes a marked reduction in the browning. Sugars therefore are concerned in the natural browning of dried fruits. At least three compounds isolated from apricots and from strawberry preserves can be directly traced to the sugar. Two of these are furfurals. These accelerate

browning when added to apricot concentrates, and are therefore to be classed among the intermediates of the browning reaction, at least to the extent that the sugars contribute to the browning.

Our procedures in brief outline were as follows: To determine the source of CO_2 produced in the browning of a model system, we mixed radioactive glucose with inert glycine, and inert glucose with radioactive glycine. These were incubated at 100°C. (simulating the conditions in Maillard's original experiments) and at 56.5° (chosen for convenience, the boiling point of acetone, simulating apricot storage work at 55°C.). In both cases, one may conclude that the major portion of the CO_2 came from the carboxyl of the glycine. This comprises 80-85% of the total, and less than 10% comes from the sugar under these conditions.

The products of the reaction have been chromatographed to determine the distribution of the radioactivity. In addition to the highly active brown pigment, a possible intermediate* has been found which derives activity from both glucose and glycine. Glycine can be regenerated by hydrolysis, whereas glucose cannot. Either the glucose has already been degraded, in which case the compound contains glycine and a glucose fragment, or the glucose is destroyed by the hydrolytic procedures employed. The latter seems inherently less probable to us at this juncture. Other possible intermediates have also been isolated and partially characterized.

The work is being continued in an effort to characterize as completely as possible the course of the reaction and the necessary control measures.

Work of the Northwestern University Group Using Model Systems

HURD:

As is well known, color is caused by absorption of part of the waves constituting white light; colored organic compounds usually possess such chromophoric groups as azo, nitroso, quinone, α-dicarbonyl, or extended conjugate unsaturation.

As background information on the browning reaction, let us begin with the familiar fact that colorless monosaccharides such as glucose or fructose and colorless amino acids such as glycine, alanine, or glutamic acid react readily to promote browning. The simple reaction product of glucose and glycine is not colored; hence any explanation of color must be sought in further products of condensation or in the concurrent reactions which are encountered.

One of the concurrent reactions to consider is that of the formation of furfural from pentoses, or hydroxymethylfurfural (HMF) from hexoses. It was established in 1946 that furfural was indeed formed by boiling a mixture of xylose and glycine solutions. Previously, others had shown that glucose, treated similarly, yields HMF. It had been shown also that HMF

* C. O. Chichester has shown that glycine can slowly be regenerated from this compound though glucose cannot. The compound can be hydrolyzed to give H.M.F. Thirty-five milligrams of this compound have been separated on a cellulose column. It is free from glucose, but a small percentage of glycine is present as a contaminant. The compound is colorless, but darkens readily on warming. It is levorotatory, with a minimum $[\alpha]_D$ of −40°. Additional properties will be determined.

was present in browned fruit juices. In view of this, one needs to inquire whether these colorless furfural bodies are dominant or incidental compounds in the browning reaction. It has not been easy to get a decisive answer on this point, but the best evidence seems to be that they are not dominant.

Pyruvic aldehyde falls in the same category. That it can arise during the interaction of glucose and glycine has been demonstrated in Dr. Speck's work (pp. 29-36, this monograph) but whether or not it is dominant awaits better evidence than is currently available. If pyruvic aldehyde should prove to be an essential precursor, the problem of color still remains.

While not neglecting the glucose-glycine reaction, it was believed that model compounds would be advantageous in this study. Accordingly, 2,3,4-trideoxy- and 3,4-dideoxy-aldopentoses (I) were prepared and tested. The former shows very little coloration with glycine but the latter colors more rapidly than glucose. A small amount of tetrahydrofurfural was obtainable from this reaction but the brown substance itself resisted characterization.

The preparation (I) just referred to is an α-hydroxy aldehyde. Much time was spent trying to prepare 2,4-didesoxyaldopentose, which would be a β-hydroxy aldehyde. This objective was not achieved, but it was shown that aldol, another β-hydroxy aldehyde, undergoes browning with glycine.

One seemingly promising approach was interrupted for a very unexpected reason. It was hoped that (I) and glycine ethyl ester would yield the aldehyde-amine derivative in its cyclic (pyranose) form. Then, because of the $>C-C<$ structure this should be cleavable by sodium periodate:

$$\begin{array}{cc} | & | \\ OH & NH- \end{array}$$

if so, the compound could be heated and the course of reaction followed by periodate titration. First, however, (I) was tested with periodate to gain experience. Its pyranose form should have given rise to formic acid but no formaldehyde. Formaldehyde, however, was formed in quantity, as if the ketose isomer of (I) were present instead. Since this result cast doubt on the purity or structure of (I), a detailed inquiry was necessitated. This required several months' work, with the finding that fairly pure (I) can be made if rigorous steps are taken in the purification. The glycine ester work, however, has not been resumed to date.

It is felt that this model sugar will continue to be valuable in developing this study. Among other things, it suggested that other α-hydroxy aldehydes or ketones should be studied, the simpler the better. Thus, hydroxyacetone, hydroxyacetophenone, benzoin, acetoin, propionoin and hydroxyacetaldehyde have been studied. They become very simple model ketoses or aldoses.

Work with these compounds and amino acids has revealed that heterocyclic nitrogen compounds are isolable as reaction products. Benzoin and alanine, for example, gave rise to tetraphenylpyrrole, tetraphenylpyrazine, acetaldehyde and carbon dioxide. Acetoin yielded small quantities of tetramethylpyrazine but seemingly none of the tetramethylpyrrole. acetoin, ace-

tol (hydroxyacetone), and hydroxyacetaldehyde gave very dark brown solutions.

2,5-Dimethylpyrazine was isolated in low yields in the reaction of alanine on acetol. It was established that this compound was not involved in browning, since mixtures of it and acetol do not form colored materials rapidly enough.

2,4-Dimethylpyrrole might have been obtained from alanine and acetol but no direct evidence for it was obtainable. It was found, however, that this pyrrole reacts rapidly with acetol at 100° to yield brown products. Coloration was noticed even at 20°.

The alanine-acetol brown product is not identical to the dimethylpyrrole-acetol brown product but the resemblances are sufficiently close to lend encouragement to the approach. The experiments carried out to date, however, have been negative in establishing whether or not pyrrole nuclei are present in the brown products. Mercuration seemed promising at first but no clean-cut derivatives were obtained. It was hoped that oxidation methods would have yielded pyrrolecarboxylic acids or maleic imide but this approach also was not productive. It was interesting to find that reduction (Zn and acetic acid) of the brown solutions changed their color to yellow. The brown reappeared on standing.

Another variant was introduced in other experiments, namely, to use *p*-aminophenylacetic acid as the amino acid for the browning. It gave intense browning, and the hope was that the phenyl group might assist as a tracer nucleus in attempting to follow the course of the reaction. In contrast, *p*-aminobenzoic acid gave no browning. Further work on *p*-aminophenylacetic acid has been interrupted to make way for the experiments with acetol, acetoin, etc., but it will be resumed in the near future.

As a working hypothesis it is suggested that the initial step in browning is reaction of the aldose with the amino group. Much of the product then rearranges to a ketose, the amino group remaining on carbon no. 1. This ketose may cyclize to form a furfural derivative, or to a pyrrolecarbonal derivative by reaction with more of the amine. Either one would be an aldehyde (or ammono aldehyde) capable of condensing with the abovementioned ketose to form an alpha diketone. This might be one source of the color which is noticed. Since pyrrolecarbonals are known to condense with pyrroles to form dipyrromethenes and compounds of higher complexity this would be another plausible source of color. Enough support for these steps has been found to make them mildly attractive at present, but absence of any clinching evidence means that new data are required.

One may wonder whether any of these experimental approaches have anything to do with browning in foodstuffs. It is believed that they do. It is hoped that the synthetic brown products will respond to treatment in a manner that becomes intelligible. When that time comes it will be interesting to try the same procedures on the brown concentrates from a food source. If the correlation is good, there will be reason to believe that the work is on the right track.

Work of the Colorado State College Group
The Control of Non-Enzymic Browning in Potatoes

PYKE:

Several features of the process developed for the control of non-enzymic browning during the dehydration and storage of dehydrated potatoes should be of interest to the conference, and we shall briefly review them for you.

Essentially, the process controls the browning reaction by an extraction process which removes the reactants. The browning potential of the potato sample will depend upon the variety and past history. If the Rural and Bliss Triumph are compared as far as browning potentiality is concerned, the Rural would be classed as a low browner while the Triumph is a high browner. Potatoes kept in cold storage for considerable periods develop the maximum browning range for the variety in question. Nevertheless, we have found that it is feasible to remove the browning reactants from potatoes taken directly from cold storage for both high and low browning varieties. The quantity of browning reactants removed depends upon the temperature of the extracting medium, the duration of the extracting procedure, and presence of certain electrolytes.

Fortunately, it is possible to estimate rather accurately in a few minutes the browning potential of any given samples. The deep-fat frying method used for this estimation has previously been described. Briefly, the color development is accomplished by frying in Primex at 187°C. for two and one-half minutes. It is important that the frying time used bring about the complete dehydration of the potato pieces used. When the browning obtained is to be reduced to a numerical value, the fat is removed from the crushed fried samples by extraction with chloroform. The crushed and chloroform-extracted potato sample is pulverized and the developed brown color is extracted by 50-50 alcohol-water. The optical density of the brown color is estimated from the filtered alcohol-water extract which had previously been brought to standard volume.

In this manner the efficiency of the extraction process for the control of browning can be followed rather accurately. For illustration, samples prepared for dehydration from potatoes directly from cold storage may be placed in the extracting bath and subsampled from the extraction process at, say, two-minute intervals up to and including a twelve-minute interval. When the extraction process is operated at 90°C. we found that the twelve-minute extracted sample is practically white. Those samples that have undergone extraction for shorter time increments would range darker in a perfect gradation until the full depth of color is realized in the sample that has undergone no extraction.

In this series we have been describing, the quarter die is the cut used. Its dimensions are $^{3}/_{8}$" x $^{3}/_{8}$" x $^{3}/_{32}$". We have samples for those who are interested showing the application of the extraction process to Rural, Katahdin, and Bliss Triumph varieties of potatoes. These samples were removed directly from cold storage for processing.

Our experience with the extraction process shows that the removal of the browning reactants from potatoes follows the diffusion equation within the limits of experimental error. When all of the items in the diffusion equation that may readily be held constant are so controlled, it appears that the extraction rate should vary as T/n, where T is the absolute temperature of the extracting bath and n the viscosity. When the influence of T/n is considered, it would be expected that the rate of diffusion or extraction would treble between 13° and 60°C. But it would not be expected to double between 60° and 100°C. These predictions are in very close agreement with experimental findings.

The influence of electrolytes on the rate of extraction follows a cationic lyotropic series. In other words, both the rate of extraction and the total amount extracted become greater with polyvalent cations present in the extracting medium. Hot water will do a good job of extraction. However, the presence of calcium or magnesium ions makes the process much more efficient and improves the resistance of the product to abuse. The concentration of calcium or magnesium ions should range between 0.0175 and 0.035 gram ions per liter of extracting medium which is normally a hot water solution.

The rate of extraction—and consequently the economics of the process—is related to the thickness of the slice along its smallest dimension. The samples we have been discussing are the quarter dice, $\frac{3}{8}''$ x $\frac{3}{8}''$ x $\frac{3}{32}''$ slice. These are processed within a reasonable length of time. But we have found in regard to the slice which is $\frac{1}{16}''$ in the smallest dimension that extraction times are cut by about one-third. This slice is equally resistant to abuse—an important practical consideration.

It might be emphasized at this point that resistance to abuse of these dehydrated slices is wholly satisfactory. When sealed in ordinary tin cans and tumbled for hours, practically no breakage of pieces occurs. Although the entire inside of the cans will have a thin deposit of potato dust after such an experiment, this is only the result of the rubbing which the surfaces of the pieces receive during the tumbling process. In no case have we found breakage to exceed 3% of the sample. With water-extracted samples in the absence of calcium ions the resistance to abuse was much lower.

As far as reconstitution and cooking of these dehydrated samples are concerned, they appear to us to be satisfactory. When reconstitution and cooking are combined, as they may be on occasion, we have found that dehydrated samples may be prepared for the table as quickly as fresh raw potatoes are. In fact, in most cases the preparation time is much shorter than that required by fresh potatoes. Potatoes processed in this way may be prepared in virtually all of the usual forms for table servings. With the exception of whole baked potatoes, they adapt themselves readily to all the familiar potato dishes.

Another phase of our work has been to develop empirically and in a mathematical manner methods for the rapid estimation of the probable storage life of samples of dehydrated potatoes. In this development we

have made use of the deep-fat frying procedure already described and have been following rates of the browning of the samples in an ordinary drying oven at different temperature settings. It appears at the present time that a drying oven temperature somewhere around 210°F. may be selected as the most desirable for this purpose. The browning behavior at the temperature selected will then be related mathematically to other measures that have been used to estimate dehydration and storage behavior. When this work is completed, a buyer or inspector of dehydrated potatoes can determine in a few hours the storage life to be expected of the samples being considered. It is believed that he can predict rather accurately the browning behavior of these potatoes if they are to be stored at room temperature or at more elevated storage temperatures of 100°F. or 120°F.

This presentation has been brief. However, I am now going to take up for consideration observations we have made recently regarding the possibility for blocking, arresting, or inhibiting the non-enzymatic browning reaction. For these experiments rather concentrated solutions were used in contrast to the dilute solution approach. It was observed that the presence of organic mercapto-compounds having in common the sulfhydryl group prevented or greatly inhibited the development of brown color when glucose was heated with amino acids. In these experiments 5 grams of glucose, 3 grams of amino acids, and 5 ml. of water in test tubes were heated in a boiling water bath for two hours. In many cases complete inhibition of the development of brown color was obtained while controls turned almost black. It was also possible to reverse the development of brown color in partially browned glucose amino acid mixtures by addition of the mercapto-compound after browning had been in progress.

The evidence seems to indicate that the mercapto-compound reacts at a secondary stage rather than with the initial reactants added. Dr. Guss, who has been doing this work, will tell you about it in more detail, but from the observations made he has developed a theory regarding the course of the first stages of the browning reaction. This theory is both interesting and thought-provoking.

Work at Colorado State College on the Chemistry of Browning

GUSS:

A report that cysteine would inhibit browning was the impetus for looking further into the browning problem. It had been suggested that cysteine would tie up the glucose in some way, perhaps by a ring formation involving the SH and NH_2 groups. We were rather interested in knowing exactly what was responsible for this inhibition by cysteine. Actually cysteine will brown with glucose itself, but when one takes a glucose and glycine system and adds cysteine to it, one does get some retardation, apparently, in browning. We decided then to try sulfhydryl compounds. Into a test tube containing 5 cc. of water we put 3 g. of glucose and about 0.5 g. of an amino acid. We used either glycine, sodium glutamate or lysine hydrochloride. Putting this under a reflux condenser, we heated it in a water bath at 95°C. for two hours. At the end of this time the reaction mixture was a dark

brown. It would darken to a light yellow at first, then to a darker yellow, then to a sort of reddish color, then to a reddish brown, and finally to nearly black. Addition of three drops of certain sulfhydryl compounds prevented color formation under these conditions. No browning occurred, at least no brown color appeared. This result depended somewhat upon the amino acid and the sulfhydryl compound used. We do not believe the sulfhydryl compounds react with the glucose or with the amino acid. Solutions of glucose and amino acid plus the sulfhydryl compound, such as beta-mercaptoethanol, beta-mercaptopropionic acid, mercaptoacetic acid, BAL, or other water-soluble mercapto compounds, exhibit little or no browning. Sodium bisulfite, sodium hydrosulfite, thiourea, and, to some extent, other sulfhydryl compounds will inhibit browning. When we added a mercapto compound to glucose or to glycine, we found that there was no decrease in the concentration of glucose or glycine after heating for long periods of time (5½ hours) at reflux. Now, unless there was an easily reversible reaction going on, one cannot conclude that there was a reaction between the sugar or the amino acid and the sulfhydryl compound. Blocking does not occur at the beginning of the reaction; it appears rather to occur near the end. If one does not brown a mixture, such as glucose and glycine, too far—say, to the point where one gets a yellow or reddish-brown color—and then if one adds a sulfhydryl compound to this mixture, the color fades. It doesn't disappear completely, but one can make it fade back. This is known to occur with sodium bisulfite, too. This demonstrates that you can cause the reaction to go to a colored stage, and that one can then reverse it back to a non-colored stage. However, if the reaction has gone too far, it cannot be reversed—not completely at least.

Now, the people who are interested in furfural will probably be interested to learn that furfural plus glycine in the presence of a mercaptan shows no inhibition of browning. This mixture browns just as fast as when the mercaptan compound isn't there. But if one takes glucose plus glycine and adds a small amount of some mercapto compound, complete inhibition of browning can be achieved.

This makes one wonder whether furfural is ever formed in the reaction of glucose and glycine to give brown products. If furfural is formed it will certainly brown with amino acids, there is no doubt about that. One can take a sugar, such as glucose, and aminoethanol and get a change of color exactly like a browning reaction gives. Mercapto compounds do not inhibit this color formation. Perhaps the inhibition of a browning reaction by a mercapto compound may be diagnostic of the type of browning that one gets in the sugar-amino acid systems. It suggests that one may be able then to exclude some of the other so-called browning reactions from this category. The practice of putting two materials together to get a brown coloration seems to me of somewhat dubious value. One can do that with a great many substances—too many. One can get very far afield from the reaction of glucose with glycine in this way.

It has been observed that one can use up all of the sugar and amino acid and still not reach the colored stage. We can agree with that. One can per-

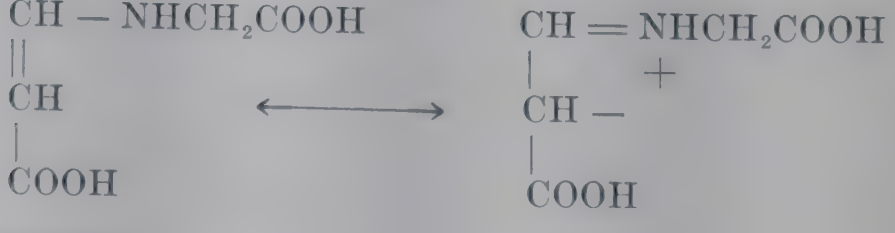
haps put glucose and glycine together and not have any color formed, but one may not have any glucose or amino acid present as such in the solution either. Apparently the mercapto compound is acting at some point close to the end of the colorless stage.

What are the requirements of a glucose-like compound that will still give a brown color and perhaps still be inhibited by a mercapto compound? To shed light on this point, we tried many compounds. For example, lactic acid with an amino acid doesn't brown, but pyruvic acid does brown with sodium glutamate. One can move the CO group farther from the COOH and still get browning. By using ethyl acetoacetate (not the free acid) or levulinic acid you have moved the CO out farther and both will brown. One can get browning with aldol. You had verification from Dr. Hurd on that—aldol will brown. Some others such as lactose and maltose will brown. Biacetyl is a very good browner—in fact, it browns very rapidly. We tried dihydroxymaleic acid, which browns very rapidly with CO_2 evolution. We tried all of the aminobenzoic acids, and they all brown under our conditions. We feel certain that we can put para-aminobenzoic acid from an Eastman bottle of para-aminobenzoic acid with some glucose and water and heat it up to 95° for two hours and it will be quite dark. This is inhibited by beta-mercaptopropionic acid, a sulfhydryl compound. One can get browning with anthranilic acid. The aminobenzoic acids will all brown and they are all inhibited by mercaptoethanol.

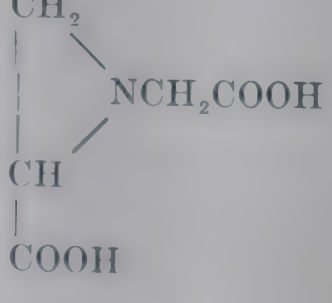
It is unfortunate that repetition of some work reported in the literature must be done because of the difference in conditions employed by various workers. Apparently the conditions used are of real importance in determining whether or not one will get browning.

We found that beta-alanine browns faster than alpha-alanine. It would seem that one does not need a carboxyl group next to the amino group.

What I am going to say now is highly speculative, but it will give you some idea of the trend of our thinking. If one starts with the enolic form of, say, pyruvic acid, it is possible to go through a sequence of manipulations which are not too ridiculous and arrive at, say, an allylic system:



or an ethylenimine:



Such structures ought to be polymerizable, and we feel that a polymerization is involved in the browning reaction. The observed evolution of CO_2 could also be explained, although we feel that CO_2 evolution is not a requisite for browning. It now appears to us that an enol structure must be a potential feature of the structure of the carbonyl compound in order for browning to occur. We do not believe that the carbonyl of a sugar is necessary, nor do we feel that furfural or hydroxymethylfurfural is a necessary precursor in browning. We do not believe that an anil or Schiff base type of linkage, $-CH{=}NCH_2COOH$, is an intermediate in the browning reaction.

The Browning Program at Michigan State College

SPECK:

The probability that the degradations of reducing sugars and amino acids, which occur upon heating aqueous mixtures of these two classes of substances, contribute significantly to the non-enzymic browning of foods had already been recognized at the time this investigation was begun, for certain of the general characteristics of these reactions, such as melanoidin formation and decarboxylation of the amino acid, had been observed many years previously by L. C. Maillard *(1)*. Nevertheless, the nature of the reactions by which these substances are degraded and the catalytic factors involved in these transformations, not to mention the structures of the intermediate and end products, were almost entirely unknown. It was therefore generally agreed that the group investigating the fundamental nature of browning should, for the most part, confine its attention to model systems composed of amino acids and reducing sugars in an effort to elucidate the chemistry of this Maillard reaction.

EARLY EXPERIMENTS

The first attempts in this laboratory to determine the nature of the reactions occurring in such model systems involved examination of their spectra. It soon became apparent, however, that the transformations which take place in even relatively simple mixtures, such as those composed only of glucose and glycine, were far too complex to permit analysis of spectral changes. In revising the approach it was decided to attempt the isolation and characterization of the products formed under these conditions from simpler reducing sugars. The first of these experiments were carried out with aqueous mixtures of the trioses (glyceraldehyde and dihydroxyacetone) and several simple amino acids. These were distilled and examined for volatile products. The optical densities of the residues were also determined at 440 mμ. The results may be summarized as follows (see also Tables 1 and 2): (1) Both glyceraldehyde and dihydroxyacetone browned rapidly when heated with amino acids such as glycine or alanine, the relative order of color formation for the glyceraldehyde mixtures with different amino acids being: β-alanine > glycine > d,l-alanine. Practically no color formation occurred where N,N-dimethylglycine was the amino acid component. (2) Pyruvaldehyde was identified among the volatile products from the re-

action mixtures which browned, after isolation in fair yields as its disemicarbazone. None was isolated from the N,N-dimethylglycine mixtures. (3) Aldehydes which would be expected from the oxidative deamination-decarboxylation of α-amino acids in these mixtures were also identified among the volatile products.

TABLE 1

Amino Acid-Glyceraldehyde Distillations

Amino Acid	Pyruvaldehyde Yield, %
Glycine	10.1
d,l-Alanine	5.65
β-Alanine	2.78
N,N-Dimethylglycine	0.0

TABLE 2

Mixture	Optical Density 440 mμ
Glycine-Glyceraldehyde	0.922
d,l-Alanine-Glyceraldehyde	0.628
β-Alanine-Glyceraldehyde	1.12
Glycine-Pyruvaldehyde	0.051
d,l-Alanine-Pyruvaldehyde	0.036
β-Alanine-Pyruvaldehyde	0.084
N,N-Dimethylglycine-Pyruvaldehyde	0.006

These observations led to the conclusion that the trioses are capable of undergoing a typical Maillard reaction in the presence of amino acids having a primary amino group. They also brought into sharp focus the substance pyruvaldehyde which had been postulated as an intermediate in the browning reaction by various investigators *(2)*. Tests carried out with mixtures of pyruvaldehyde and glycine, β-alanine, and d,l-alanine showed that these mixtures browned rapidly. Again the order of color formation was β-alanine > glycine > d,l-alanine. No browning was observed for mixtures of N,N-dimethylglycine and pyruvaldehyde, and the recovery of pyruvaldehyde from this mixture was relatively high (Tables 2 and 3). In

TABLE 3

Amino Acid-Pyruvaldehyde Distillations

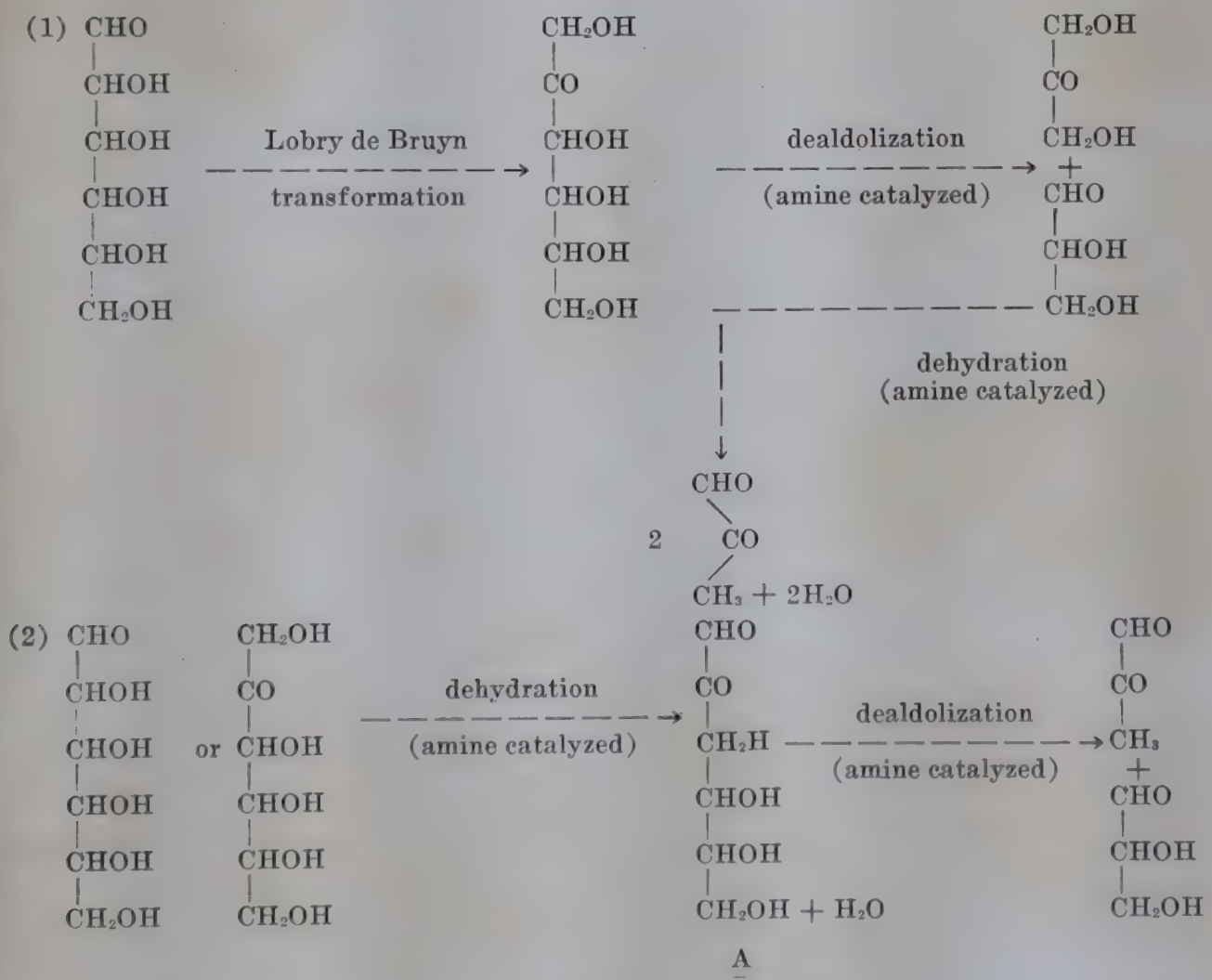
Amino Acid	Pyruvaldehyde Recovery, %
Glycine	19.5
d,l-Alanine	13.5
β-Alanine	11.3
N,N-Dimethylglycine	34.8

view of these results it seemed probable that amino acids having primary amino groups catalyzed not only the formation of pyruvaldehyde but also its polymerization via successive aldolizations. Aldolization is known to be subject to catalysis by primary and secondary amines, and, in a rough quantitative check on the relative catalytic efficiency of the amino acids used in the previous experiments for the dealdolization of diacetone alcohol the following order was observed: β-alanine > glycine > d,l-alanine.

In extending this method of investigation to mixtures of amino acids and higher reducing sugars evidence was obtained for production of pyruvaldehyde, the corresponding furfural and a new (for these reaction mixtures) α-dicarbonyl compound, diacetyl.

As a result of these early experiments the following working hypothesis was adopted:

(a) Pyruvaldehyde is produced during the reaction of a higher reducing sugar with an amino acid by one or both of the reaction sequences indicated below:



(b) Compounds represented by structure *A* are further dehydrated to furfural, or substituted furfurals.

(c) Oxidative deamination-decarboxylation of α-amino acids in these mixtures results from the interaction of these substances and α-dicarbonyl

compounds (pyruvaldehyde, diacetyl, and compounds represented by *A*, or its analogues) in the Strecker degradation. This reaction is illustrated by the following idealized equation:

$$RCOCOR + R'CHNH_2CO_2H \longrightarrow R'CHO + CO_2 + RCHNH_2COR$$

(d) The pigmented material formed in the Maillard reaction arises from a complex polymerization (which is partly aldolization) of the α-dicarbonyl compounds and other aldehydes, including the furfurals, produced in these mixtures.

QUANTITATIVE EXPERIMENTS WITH HEXOSES AND PENTOSES

Since very little data had been obtained in the previous experiments for a possible correlation of browning with production of α-dicarbonyl compounds it was decided that the next step in this investigation should involve determination of the yields of pyruvaldehyde and diacetyl from higher reducing sugars, as well as estimation of the amount of pigmented material formed in these mixtures. Before this phase of the work could be carried out it was necessary to devise a satisfactory method for determining pyruvaldehyde at low concentrations. Fortunately, it had been observed that chromotropic acid (4,5-dihydroxy-2,7-naphthalene disulfonic acid) gives a test with pyruvaldehyde which is apparently specific for this substance, so that a quantitative procedure could be based on this reaction. *(3)*. A new micro method for estimating diacetyl was also developed at this time *(4)* and later a scheme was devised for positive identification of pyruvaldehyde in aqueous solutions at low concentrations. The former procedure was also based on a reaction with chromotropic acid, whereas the latter depends on conversion of pyruvaldehyde to its dioxime, isolation of this derivative by extraction and paper chromatography and, finally, identification as the nickelous methylglyoximate.

The following sugars were employed for these quantitative experiments: glucose, galactose, mannose, xylose, arabinose, ribose, fructose, rhamnose and 3-methylglucose. The amino acids were glycine and β-alanine. Each of these experiments was carried out under the same conditions of concentration and heat. Where the amino acid component was β-alanine the concentrations of both pyruvaldehyde and diacetyl were determined in the distillates. Only pyruvaldehyde was estimated for the glycine mixtures inasmuch as formaldehyde from the Strecker degradation of the amino acid interfered in the analytical method for diacetyl. The color in the residues from distillations of these mixtures was measured by determining their spectra from 230 to 480 mμ. (Actually it turned out that the spectra were all quite similar, so that the optical density at either 440 or 480 mμ was considered a fairly reliable index of browning.) The results of these trials (see Tables 4 and 5) indicated no direct proportionality among different sugars between yields of these α-dicarbonyl compounds and color. For example, certain of the mixtures which appeared to produce little diacetyl or pyruvaldehyde browned much more than mixtures for which the yields of these substances were much higher. It was concluded, however, that the volatile

TABLE 4

Glycine Distillations

Reducing Sugar	Pyruvaldehyde Yield, mg/l
Glucose	0.25
Fructose	7.9
Arabinose	3.6
Xylose	3.1
Rhamnose	21
3-Methylglucose	0.09

TABLE 5

β-Alanine Distillations

Reducing Sugar	% Aldehyde (at 0.25 M)	Pyruvaldehyde Yield, mg/l	Diacetyl Yield, mg/l	Optical Density at 440 mμ
Glucose	0.024	1.3	ca. 5	0.034
Galactose	0.082	0.91	ca. 5	0.124
Mannose	0.064	1.6	ca. 5	0.093
Xylose	0.17	4.0	16	0.885
Arabinose	0.28	4.8	14	0.662
Ribose	8.5 (0.1 M)	2.4	32	1.19
Fructose		11.0	ca. 5	0.041
Rhamnose		24.0	49	0.093
3-Methylglucose		0.73	....	0.038

α-dicarbonyl compounds do contribute to the formation of pigmented material in the mixtures and that this contribution arises in part from copolymerization of pyruvaldehyde and diacetyl with either furfural or hydroxymethylfurfural. This conclusion was based on the following observations: (1) Color formation was for almost every mixture directly proportional to the percentage of acyclic, or aldehyde, form of the reducing sugar in equilibrium with cyclic forms. (2) Mixtures containing rhamnose, from which the substituted furan would be the relatively unreactive methylfurfural, gave much higher yields of pyruvaldehyde and diacetyl than those containing the other sugars, but produced little color.

In continuing this phase of the work it was decided to investigate further the volatile products from reducing sugar-amino acid mixtures in order to determine whether or not substances such as acetol or glycollic aldehyde are produced under these conditions. The procedure which was followed consisted of carrying out distillations as previously described, on a considerably larger scale, and determining pyruvaldehyde by reaction with chromotropic acid, and diacetyl by precipitation of the nickelous dimethylglyoximate. Total substances oxidizable by periodate were then determined and this data compared with the diacetyl and pyruvaldehyde values for the same distillate. Distillates from the following reaction mixtures were examined: fructose-glycine, glucose-glycine, xylose-glycine, xylose-d,l-alanine and xylose-β-alanine. The data are given in Table 6. With the exception

TABLE 6

Amino Acid-Reducing Sugar Distillations

Mixture	Pyruvaldehyde mmol/l	Diacetyl mmol/l	IO_4^- consumed me/l	Calcd. IO_4^- me/l
Fructose-Glycine	0.10	0.32	1.41	0.84
Glucose-Glycine	0.04	0.37	1.08	0.82
Xylose-Glycine	0.04	1.57	4.16	3.22
Xylose-d,l-Alanine	.....	0.19	.....	.....
Xylose-β-Alanine	0.04	0.14	4.04	0.36

of the xylose-β-alanine mixtures, the amounts of periodate reduced were 25 to 50 per cent higher than those calculated from the pyruvaldehyde and diacetyl values, indicating the presence of small amounts of compounds such as acetol or glycollic aldehyde. For the xylose-β-alanine mixture the periodate consumed was almost the same as that for the xylose-glycine mixture; however, this was ten times the calculated amount since relatively little diacetyl was present. The exact nature of the substance, or substances, in the β-alanine distillates which are responsible for this discrepancy has not yet been elucidated.

EXPERIMENTS ON THE CATALYSIS OF DEALDOLIZATION

The nature or magnitude of amino acid catalysis of dealdolization had not been determined prior to this investigation. Accordingly, catalytic constants were evaluated kinetically for glycine, d,l-alanine, β-alanine and glycylglycine buffers in the dealdolization of diacetone alcohol. The results demonstrated that the catalyst in each of these systems is the anion and not the zwitterion. It might be assumed that the anion functions as a base if it were not for the fact that this dealdolization is *not* subject to general base catalysis. Hence, it can only be concluded that anions of amino acids, or peptides, function as amine catalysts in dealdolization. Values for the catalytic constants are given in Table 7. The magnitude of the catalytic

TABLE 7

Catalytic Constants for Amino Acid Anions in the Dealdolization of Diacetone Alcohol

Catalyst	$k \times 10^4$ mol^{-1} min^{-1}
β-Alaninate	26.4
Glycinate	18.5
d,l-Alaninate	8.1
Glycylglycinate	8.3

constant observed for the β-alaninate ion was approximately one-twentieth that for methylamine in this reaction. Upon examining glycine buffers for augmentation of the catalysis by magnesium and cupric ions no such effect was found. Instead, diminution in the rate occurred.

AMINE CATALYSIS OF THE DEGRADATION OF HIGHER REDUCING SUGARS

Experiments with the trioses strongly indicated the operation of a catalysis by primary and secondary amino groups in the degradation of these substances. For this reason a series of experiments was carried out with mixtures of xylose and glucose and amines in order to ascertain whether such effects contribute in the Maillard reaction with hexoses and pentoses. Solutions of three amines, ethanolamine, diethanolamine and triethanolamine, which had been adjusted to pH 6.0 with sulfuric acid, were used for these trials. It was found that ethanolamine brought about a drastic degradation of the reducing sugar with formation of much black, insoluble material, small concentrations of pyruvaldehyde and, in the xylose distillate, relatively large amounts of furfural. The effect of diethanolamine was similar but less marked. No browning of the mixtures containing triethanolamine occurred and, with exception of small amounts of pyruvaldehyde, no volatile products were found in the distillates. It was therefore concluded that those amines and amino acids which catalyze the formation of pyruvaldehyde and the furfurals from the higher reducing sugars do so through participation of primary or secondary amino groups.

ACID-BASE CATALYSED TRANSFORMATIONS OF THE TRIOSES

Although the previous experiments indicated a specific amine catalysis for conversion of the trioses to pyruvaldehyde, as well as for analogous reactions among the higher sugars, they did not preclude the possibility of general acid and base catalysis of these transformations. Therefore it was decided to make a thorough examination of the kinetics of triose degradations in order, possibly, to reveal the magnitude of general acid and base catalytic effects. Again it was necessary to develop satisfactory analytical schemes for determining the concentrations of the trioses, which proved to be interconvertible under these conditions, and their common dehydration product, pyruvaldehyde. The analytical procedure which was finally employed depends entirely on periodate scission. Starting with a known glyceraldehyde concentration the concentration of glyceraldehyde can be found at any time by simply determining periodate reduced by an aliquot of the solution, regardless of whether the final product is dihydroxyacetone or pyruvaldehyde, or both. The total triose concentration can then be determined by periodate oxidation and estimation of the formaldehyde produced. An improved method for determining formaldehyde in periodate oxidation mixtures was developed for this purpose. From the total triose value the dihydroxyacetone concentration is obtained by subtracting the glyceraldehyde concentration, whereas the pyruvaldehyde concentration is found by subtracting the total triose value from the starting glyceraldehyde concentration.

These experiments were carried out with both glyceraldehyde and dihydroxyacetone as the starting triose and with formate, acetate and trimethylacetate buffers as catalysts. A series of runs was also carried out in perchloric acid. The results demonstrated that both the Lobry de Bruyn

transformation and the dehydration are subject to catalysis by acids and bases. Bases such as acetate ion are more effective as catalysts than the corresponding acids, whereas the hydronium ion is a relatively poor catalyst for these transformations. The magnitude of the general acid and base catalysis of triose conversion to pyruvaldehyde was considerably smaller than that probable for amine catalysis of the reactions. There was no measurable catalysis by water or hydroxyl ion under the conditions of these experiments.

SUMMARY OF CONCLUSIONS

It is now clear that one of the principal functions of an amino acid in the Maillard reaction is that of a catalyst. The major catalytic effects by these substances which have been observed so far are almost certainly examples of specific amine catalysis and, as such, probably involve interaction of primary or secondary amino groups and carbonyl groups. Two general reactions which appear to be involved in amino acid-reducing sugar degradation and which are subject to this kind of catalysis are: (a) dehydration reactions, such as the conversion of the trioses to pyruvaldehyde, and (b) aldolization (or dealdolization). The exact contribution of these transformations to the Maillard reaction has not been ascertained, since evidence for their involvement is at present only circumstantial.

On the basis of positive identification of pyruvaldehyde and diacetyl from degradations of higher reducing sugars by amino acids, it appears likely that these substances are intermediates in both the formation of melanoidins and the destruction of amino acids in these mixtures. Nevertheless, the evidence for this is again purely circumstantial, and the extent of their participation must be established by further experimentation.

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Work at The Ohio State University

The Fundamental Nature of the Browning Reaction

WOLFROM:

This study concerns the fundamental nature of the reaction between α-amino acids and reducing sugars leading to the formation of colored polymers with the concomitant release of carbon dioxide. This reaction is known as the Maillard *(1, 2)* or non-enzymic browning reaction and is of interest in the processing of foodstuffs, especially dehydrated fruits and vegetables.

For purposes of simplification and ready study we have employed dilute aqueous solutions of a reducing sugar and a free amino acid under homogeneous reaction conditions. It is recognized that this may be removed from practice, but after the fundamentals are known the effect of high reactant concentration may be evaluated.

In most of our work we have employed an aldose, generally D-xylose, and a single α-amino acid such as glycine. It is to be noted that the latter is a dipolar ion, $\overset{+}{N}H_3 - CH_2 - CO_2^-$, and that the former is a complex tautomeric system in water. The first naive interpretation of the reaction as a carbonyl-amino interaction of the Schiff base type has been shown to be too simple *(2, 6)*.

$$\overset{H}{R\text{-}C} = O + H_2N\text{-}R \rightleftharpoons R\text{-}CH = N\text{-}R + H_2O$$

In aqueous solution the equilibrium is far to the left. It is not denied that such a reaction may be a transient step in a complex sequence. The situation may be different in an intact protein containing free basic groups. Recent work employing paper chromatography shows that plant juices contain many free amino acids.

The pH dependency of both coloration *(6, 7)* and carbon dioxide formation *(9)* follows the curve of Figure 2. This shows four definite regions: strong basic catalysis between pH 6-8 (5-6 is the isoelectric point of glycine) for the rate of coloration (rate of carbon dioxide evolution not measured); slight basic catalysis for the rate of carbon dioxide evolution be-

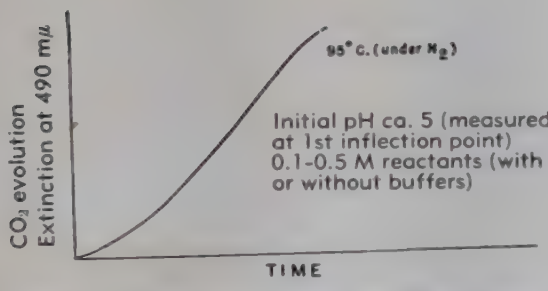


FIG. 1. Rate of coloration of carbon-dioxide evolution; aldose—α-amino acid.

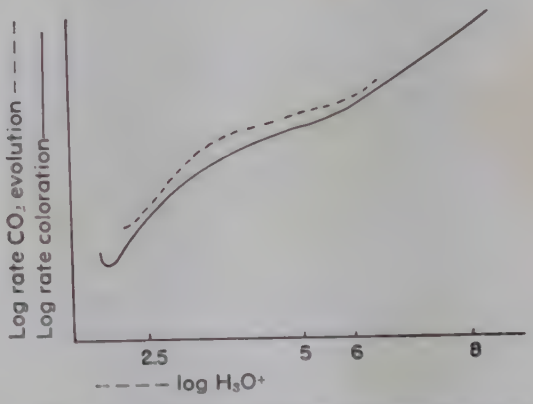
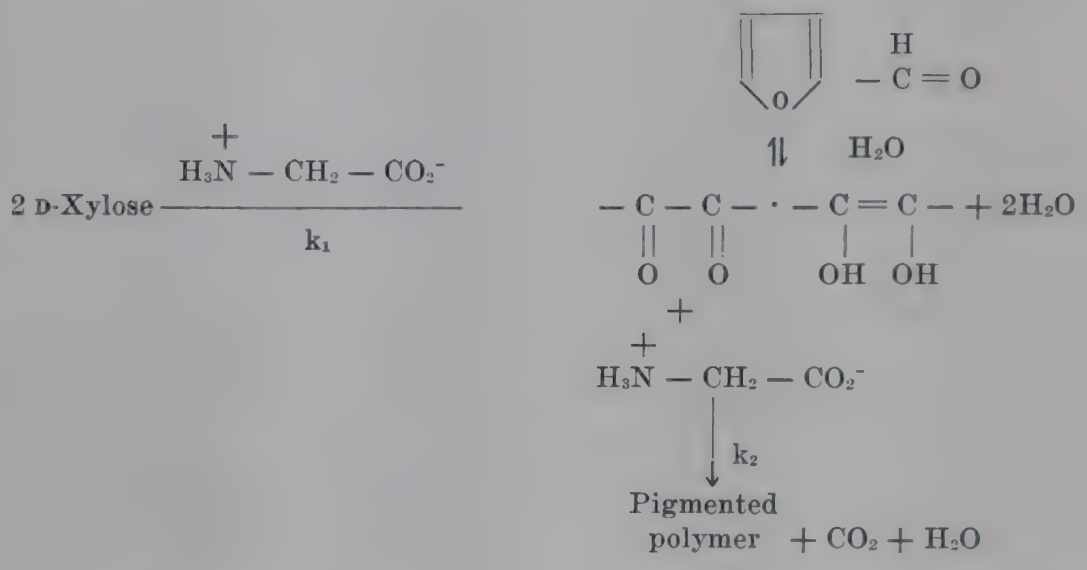


FIG. 2. pH dependence of reaction between d-xylose and glycine (experimental conditions as in Figure 1). pH (initial) was measured at flex point, no buffer employed.

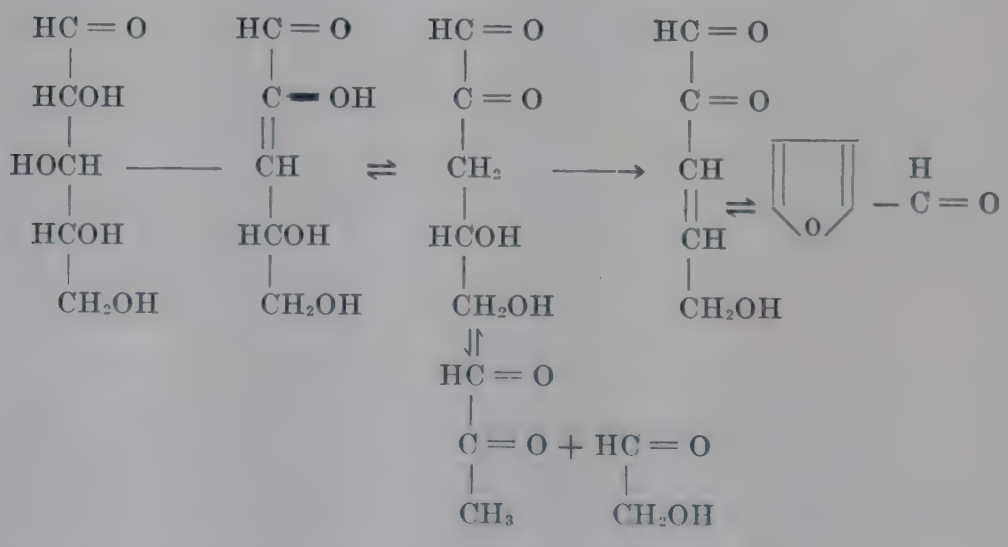
tween 2.5-5 for both (rate of coloration not well defined here because of blue-green off-color); acid inhibition below 2.5 with an upturn at very low pH probably indicative of heavy 2-furaldehyde polymerization. The formation of 2-furaldehyde from pentoses *(6)* and of 5-(hydroxymethyl)-2-furaldehyde from hexoses *(5)* under acid catalysis has been studied by absorption spectra and intermediates (unsaturated carbonyl compounds) established.

Tracer experiments with C^{14}-labeled glycine show that the carbon dioxide originates in the carboxyl group of the glycine *(9, 10)*. Under nitrogen and at the pH 4-5 of the D-xylose-glycine system, one mole of carbon dioxide requires for its formation two moles of D-xylose and the reaction stops at this point *(9)*. In the presence of oxygen (air) the sugar can de-

carboxylate an excess of glycine *(9)*. We are therefore dealing with a Strecker *(11)* type of degradation involving a redox system produced from the sugar by dehydration with or without fragmentation. The induction period no doubt represents the time required to build up an effective concentration of these sugar decomposition products.

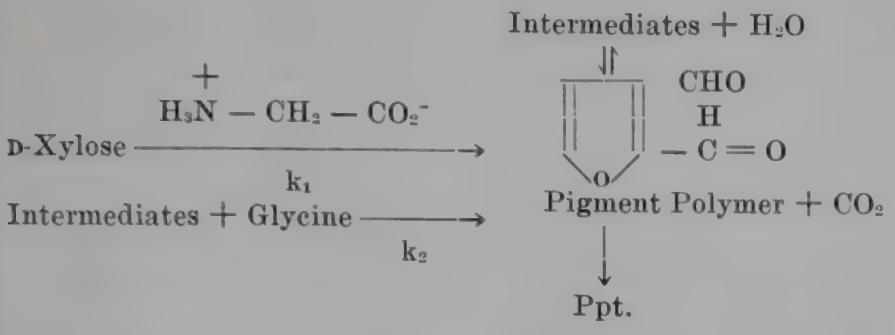


The intermediates may be formed by dehydration without chain scission or by scission through dealdolization. At pH 5 the former is presently favored (2 :1 ratio of D-xylose : CO_2, under nitrogen) but both mechanisms may overlap and probably involve the same intermediates.



The reaction, as measured by either the rate of carbon dioxide evolution or by the rate of color formation (extinction at 490 mμ), follows a sigmoid curve (Figure 1) *(8, 9)*. The induction period present is indicative of the formation of reactive intermediates and may be eliminated *(6)* by the addition of 2-furaldehyde or 5-(hydroxymethyl)-2-furaldehyde, small amounts of which are formed *(5, 6)* in this reaction from the sugars, a pentose yielding the former and a hexose the latter. At the same time it is recognized

that these furan bodies are not necessarily the true intermediates but may be a "resting stage" in equilibrium with them *(7)*.



The glycine exercises a slight promoting effect upon the formation of furan bodies *(6)*. The kinetics of the second reaction (k_2) may be measured by the slopes of the flex tangents of the sigmoid curves for carbon dioxide evolution or color formation. It is second order or first order with respect to each reactant *(9)*.

a (xylose)	*b* (glycine)	E/min.	(CO_2) min. × 10^5
0.250	0.250	0.011	
0.500	0.250	0.020	
0.250	0.500	0.021	
0.125	0.125		1.0
0.125	0.250		2.1
0.250	0.125		2.4
0.250	0.250		4.4

The data show that carbon dioxide and color formation parallel each other. Furthermore, certain conditions that influence the one influence the other and to the same extent *(9)*, thus:

Buffer effect: (Phthalate)	pH	E/min.	Ratio	(CO_2) min. × 10^6	R Ratio
0.0	4.6	0.00400	1.0	6.1	1.0
0.050	4.6	0.0105	2.6	18	2.9
0.250	4.9	0.0267	6.7	44	7.2
Dimedon effect: (Dimedon)					
0		0.0105	3.0	17	3.0
0.25		0.00356	1.0	5.7	1.0

O-Methylation on C-3 does not stop browning at pH 5 or 7.5 (evidence against fragmentation between C-3 and C-4) *(4)*. *O*-Methylation on C-2 stops browning at pH 7.5 but does not have much effect at pH 5 (demethylation may occur) *(4)*. Two *N*-methyl groups on the amino acid stops browning *(4)*.

The chemical nature of the polymer and how it is formed is little understood. Some work on the water-soluble non-dialyzable fraction has been

carried out *(7, 9, 10)*. It is acidic, unsaturated, and contains nitrogen. Tracer experiments with C^{14} show that it entrains the C-1 of the aldose and at least some of the methylene carbon of the glycine; under particular conditions it may entrain some of the glycine carboxyl carbon; it contains a redox system.

Further studies will be directed toward the elucidation of these complex reactions. The nature of the intermediates and of the induction period will be further investigated. Attempts will be made to find agents that affect the latter, especially such as may inhibit it. The structure of the polymer will be further studied. Tracer techniques will be employed. The influence of high reactant concentration will be investigated.

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Work at The Ohio State University on Color Formation in D-xylose-glycine Mixtures, at 65°C. in Nitrogen, With Varying Percentages of Water

ROONEY:

Quantities of water, varying from 15% to 90%, were added to a constant weight (5 to 1 molar ratio) of glycine and D-xylose, and the mixtures were heated for different lengths of time at 65°C., with stirring, and in a nitrogen atmosphere. The coloration values, measured at 490 mμ, are

shown in the accompanying graph. In the range from 15 to about 65% water the system was heterogeneous, while with more than 65% water, complete solution was achieved.

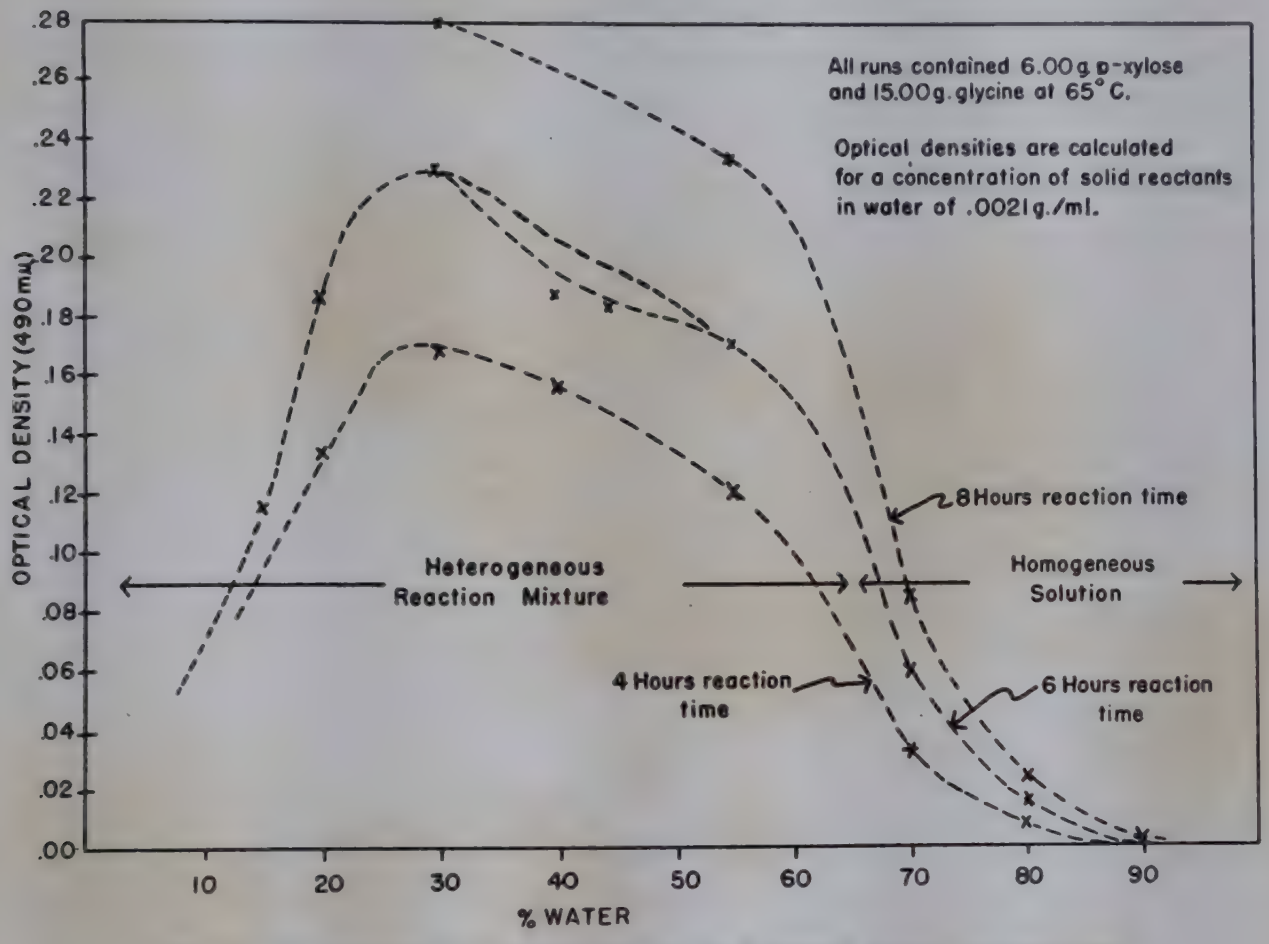

FIG. 1. Browning of D-xylose-glycine at different water percentages.

From the plot of amount of color against per cent water, the time being constant, it was found that in the homogeneous solution portion of the curve the quantity of colored polymer formed increased quite rapidly with decrease in water content below about 80% and continuing to approximately the beginning of the incomplete solution region (65% water). At this point the magnitude of the upward slope (with decreasing water per cent) lessened considerably. A maximum was reached at roughly 30% water, and beyond this value (20%, 15% water) there was a rapid decrease in the amount of coloration.

By plotting optical density against time, at various water percentages, the usual sigmoid curves were obtained in the homogeneous solution region (70, 80, and 90% water). In the heterogeneous portion (30 and 55%), when similar plots using just three points were drawn, the three values fell on straight lines which, when projected, cut the ordinate axis above the origin. The reason for this is not known. The slopes of the straight line portions were equal at 30 and 55% water, and 2.1, 5.9, and 43.0 times greater, respectively, than those at 70, 80, and 90%.

While precise kinetic interpretation of these results is not possible, the important effect of water concentration on browning was demonstrated. It

is well known, of course, that the rate of browning in general is very slow at both low and high moisture contents.

Products from D-*xylose-glycine-dimedon mixtures*

From the interaction of D-xylose with glycine in the presence of dimedon products were isolated which were not produced by the action of dimedon alone on D-xylose. By means of column chromatography several different fractions, containing no nitrogen, were obtained. These compounds are undoubtedly derived from the D-xylose, and there is some evidence that 3-deoxy-D-xylosone may be involved.

III. Discussion

MITCHELL:

In this review of a highly complicated subject which we have just concluded there have been presented the methods used in attacking the problems as well as considerable insight into product applications. The meeting is now open for general discussion, the aim of which is to consider in the light of the current status of our projects our next steps. There may be, however, a few technical questions to clear up before we begin discussing problems of future planning. Dr. Anson, I believe you have a question.

ANSON:

Dr. Lea raised a very important practical point regarding damage to nutritive value before visible browning. It was not clear to me at exactly what stage that damage occurs. Does this first compound that is formed (and which Lea has isolated) have the original nutritive value or is it some later modification of the first compound, still prior to browning, which brings about the loss of nutritive value? Actually, this is a problem of enormous importance in the animal food industry, where untold tons of animal feed have their nutritive value very seriously damaged by the browning reaction.

LEA:

In a few days when an amine-glucose mixture is stored at 70°C. and provided your moisture content drops, there is a loss of biological value in the protein. Dried milk is normally stored at a sufficiently low moisture content so that this reaction is pretty slow. In high moisture content dry milk (about 7% moisture in the skim milk powder and about 5% or a little bit more in dry whole milk) this reaction proceeds rapidly. It proceeds more rapidly at high temperatures and conversely much more slowly at moderate temperature such at 5°C.

In the case of milk powders we found a definite deterioration in flavor before any appreciable amount of lysine had been combined. In feed stuffs the moisture content may be nearer the optimum, in which case the reaction may go a lot further.

A considerable amount of lysine is combined before you get any appreciable loss in biological value. In casein, lysine is not a limiting factor in the retention of biological value, the limiting factor is methionine. Therefore you have to destroy a lot of the lysine before you begin to get a loss in biological value.

MITCHELL:

Dr. Hurd, a question was raised as to loss of nitrogen from the glucosamine. Could you elaborate somewhat on this point?

HURD:

In regard to the loss of nitrogen from the glucosamine, as in the casein experiment, that might be very beautifully explained by the process of the

Amadori type where your carbon on atom 2 that holds the NH_2 group becomes potentially a ketone type of carbon—(in which the nitrogen is rather easily hydrolyzed).

On another point—in our work with acetol, which is simply hydroxyacetone, we used an amino acid (phenylalanine). The only comment to be made in this regard is that in the oxidation of the brown product benzoic acid was isolated. This fact carries the implication of incorporation of at least a part of the carbons of the amino acid into the ultimate polymer. Therefore, if it is argued that the mixture has simply deteriorated into ammonia, there ought not to be a benzene ring persisting. Benzoic acid was actually isolated, but I am not prepared to state what it means beyond that point.

MITCHELL:

Since your work, Dr. Geddes, has led you into browning as it affects cereal products, perhaps you have some comments to make pertinent to the chemistry of browning.

GEDDES:

Our work on the browning reaction was done in connection with canned bread. We found that several changes occurred—a decrease in the reducing sugars and also a decrease in amino nitrogen in the bread. We had made certain through periodical examinations during storage, that we were not dealing with microbiological activity. At a result of our conclusion that browning was a factor in the situation, we went to model systems but studied browning in a little different type of model system from that which the organic chemist uses. We used gluten, starch, and various sugars and studied the rate of browning at different pH levels. Then we proceeded to attempt to isolate some of the substances wherein degradation products presumably were present. In brief, we found a somewhat similar situation to that found by the California group working with the browning of fruits. We obtained a whole series of carbonyl compounds, apparently; we then attempted to isolate hydroxymethyl furfural by forming the dinitrophenylhydrozones. We could not detect among these carbonyl compounds, I must add, any dimethylhydroxyfurfural. That broadly summarized our work. We felt that we should not continue with these studies until there was more basic work done to establish the techniques we had to follow. We followed, of course, some of the other changes that occur during the storage of bread, particularly the staling phenomenon.

Incidentally, I feel that the organic chemists have shown us the way to further developmental work in the product field.

MITCHELL:

Turning now to considerations relating to the profits to be derived from the organic chemistry approach, I should like to call again on Dr. Lea for any comment he may wish to make on today's proceedings.

LEA:

One thing arising from our own work is the possibility of using this method of controlling the water content of the system as one means of breaking down the browning reaction into its stages. Ascertaining the stages would benefit product applications no doubt.

It was Dr. Guss who suggested that the use of SH as an inhibitor does not block the primary reaction but seems to prevent the subsequent development of the browning degradation products. This finding might help to isolate the first product of the reaction. By control of the relative humidity —you will recall that we study these substances at relatively low humidities —one does more or less separate out the first reaction products in a reasonably clean state. It is then possible subsequently to study their breakdown.

Dr. Wolfrom in his sugar and amino acid model systems in aqueous solutions certainly seems to have evidence which indicates preliminary decomposition of the sugar and the reaction of the sugar decomposition products with amino compounds, etc. Evidence for the participation of the Strecker mechanism in decomposition seems to be very strong. The different systems we have been working with—proteins and sugars in the dry state at low temperatures and nearly neutral pH—seem to demonstrate that the first stage of browning is a simple reaction of the amino compound and the sugar. That these findings bear on the browning of products appears evident.

MITCHELL:

Dr. Hurd, may we have your appraisal of the situation?

HURD:

Browning is a problem that has two principal aspects. One aspect is a practical one—for example, how to make eggs better and better. This is a profitable objective and its attainment is important even though you cannot understand all the chemistry that is involved. The other aspect is the academic one where you put aside applications and try to find out what is happening. That aspect (1) would be helped by work related to aspect (2) is problematical, but it seems reasonable to expect that if you know what is happening you are in a better position to stop the undesirable reaction. But it is like a road block. Perhaps once you know why the cars fail to move on the blocked road, you are no better off than when you know that the highway is broken up ahead of you. However, if you are aware that the highway is impassable, you can at least plan a detour.

There are reams and reams of material that have to do with browning. The more obvious highlights are perhaps these: The browning reaction is defined as "the irreversible reaction of amino acids with some reducing sugar," at pH values between about 2 and 7; the reaction yields brown polymeric materials. Model system reactions, highly simple when compared to natural systems, can be brought closer and closer to actual reactants. As to the isolation of intermediates by this means, model systems, it must be

said that nothing has been isolated beyond the first intermediates which contain nitrogen. It would probably be informative to study compounds that function in a manner that might be studied by paper chromatography. In other words, it may be possible by this means to obtain a clue as to what is happening after the initial stages. In physical appraisals, kinetic studies have been helpful, but perhaps more thought should be given to what we are actually trying to find. When you stop to consider that the nature of the reactants and the nature of the process are both unknown, kinetics cannot be too informative because kinetic studies are quantitative. Quantitative experiments are not often helpful when you don't know the object of your study qualitatively. Nobody would think of analyzing qualitatively for iron, for example, without qualitative information.

In regard to browning, when we consider absorption spectra it looks as though we ought to take spectra on reactions as they are proceeding rather than on the final strong product. In the main the foregoing are the conclusions that can be made in a general appraisal of the problem.

MITCHELL:

Continuing our survey of points of view let me call on Dr. Mrak who has long been associated with these problems.

MRAK:

Admitting at the start that I'm not an organic chemist, I have been interested in the program for a long time through my connection with the Committee on Food Research. Along the way at some point we have lost an influence that is very important—the attempt to integrate the work along fundamental lines with that being done on commodities. In order to get the most out of this coordinated attack it is absolutely essential to seek to tie the two approaches together. The work toward one objective influences that done toward the other. In short, work on products can contribute to the work using model systems. From the standpoint of the Armed Forces, there is the money they have put into browning work over the past six or seven years. It is probable that the industry has made more use of browning data than has the Army. The Army, for example, has increased the use of SO_2 in certain commodities; the industry is more careful about that today. The industry is using selective storage if they have products—say for example, apricots—which they know are low in sulfur dioxide. In industrial practice, such products are stored in places where the average temperature is lower. In the industries, moreover, they will attempt to get rid of such products in a hurry. There are many other practices that the industry is following. They cool their warehouses at night. The many attempts to get the Army interested in taking this measure have failed. In Chicago they are interested in doing this, but red tape at some place or other has prevented and nothing has been done about it. I hope this is put into the record.

Another thing that industry has done is to take varietal differences into consideration. To exemplify the importance of this point—we have certain

peaches that will darken regardless of what you do and we have certain varieties of apricots that have actually gone out of production because of their susceptibility to darkening. The industry is paying more attention to damage sustained during dehydration. Out of one of these Army programs came the realization that there can be such a thing as heat damage in dehydration. It was found that if the product is not removed from the dehydrator soon enough—that is, if you dry at a high temperature—then you must remove the product from the dehydrator at a higher moisture content. Otherwise, incipient browning, as we call it, will take place. It is not apparent at first, but can be picked up spectroscopically. Eventually it will show up prominently and unmistakably in shorter storage life. While I'm on this point, I wonder if there isn't an indication at this early stage of some highly significant intermediates. You cannot see the discoloration; it does not show up in the extracts, and yet it shows up spectroscopically. Perhaps this is something to look at more critically.

Turning to the handling of raw material and thinking in terms of fruits—we know, for example, that if we leave prunes on the trees too long there is browning. Some of that may be enzymatic, but some of it may very well be non-enzymatic.

That brings us around to harvesting procedures. Storage of raw products at various moisture levels might be profitable, as was brought out earlier. The Western Regional Research Laboratory is working on dehydrocanned fruits with the object of having a concentrated fruit with the moisture content high enough to minimize the possibility of rapid browning.

There is one final point—somewhat apart from the central theme of my remarks. About the end of the war an attempt was made to predict the storage life of dried fruits and vegetables. The hope was that if you could run the SO_2 content on a fruit, get the degree of browning, store for about three or four days at an elevated temperature, and then run the two again, you would obtain an indication of storage life. Perhaps that is worth reviving for the practical contribution it could make to the alleviation of loss from browning.

MITCHELL:

Dr. Anson, may we have your thoughts on the problems?

ANSON:

The question has been raised whether the Armed Forces should continue to give money for the study of the straight organic chemistry of the browning reaction, a study which is somewhat removed from the practical problems.

First of all, as Dr. Mrak has said, the organic chemistry studies should not be carried on independently of work on products. And, indeed, the original program called for a balance between practical and theoretical work, and for continuous coordination of all the projects. As time has passed, however, the original projects have mostly been dropped, except for

the straight organic chemistry projects. All this more by accident than by plan. Insofar as work has been going on in dried eggs and the like, it has been done in administrative isolation of the theoretical work. That seems to me bad. If the browning program is to be continued at all, it should be the responsibility of Quartermaster leadership to see to it that the program remain properly balanced and coordinated.

It has always been agreed that if the browning reaction is of great concern to the Army and to the food industry, then the Army ought to support some general scientific work in addition to work on products. Support of basic research is common in industry, and in other fields of Armed Forces research. For instance, the Armed Forces supports work in general nuclear physics to support atomic weapon development. Now it is essential that basic work be long range if you are going to have it at all. You cannot turn profound experimental studies on and then turn them off suddenly without disrupting progress and damaging the general reputation of the Army in university circles. It may be necessary in Army-sponsored research to discontinue research due to limited funds but the research ought to be stopped in a planned way and with sufficient notice. If the Army intends to support general scientific study of the browning reaction, there has to be a certain degree of permanence in this aspect of the program. You can never get people of the quality we have here to work for the Army unless they feel that the program is established, planned for the long pull, permanent. Without stability in Army policy, scientists of quality will not become interested in a novel problem and they will not be able to attract good Ph.D. students, or, today, any Ph.D. students at all.

So much for the administrative side. On the technical side, these are two points I should like to make. When suggestions are made about mechanisms, attempts should be made to devise tests for discovering the importance of those mechanisms in the actual browning of the products in which the Army is interested. That was done in a few cases, but it has not been done nearly enough. Secondly, one of the main conclusions out of all of the study of mechanisms has been that the reactions go through intermediates which are present in relatively small concentrations. This general finding immediately suggests the desirability of greater emphasis on inhibition of browning. Such inhibition would be hopeless if the reactions were between total sugars and total amino acids. It is in the vigorous pushing to practical application of the hints provided by the studies of the organic chemistry of browning that the more theoretical part of the browning program could be more closely tied to the eventual practical aims of the Army.

MITCHELL:

And now let us have your comments, Dr. Pyke.

PYKE:

My point of view can be very briefly expressed. From the kind of studies we have carried on it should not be inferred that we are in any way against

or do not fully appreciate basic investigation. In fact, if we were asked to carry on product research without basic investigations, we would probably turn the invitation down. I think basic studies exceedingly important, but it is not for me to decide, of course, what the ratio should be. But certainly there should be an appreciable portion reserved for basic research.

MITCHELL:

May we have your reaction, Dr. Speck? Perhaps you would like to include a brief comment on the "manpower" situation.

SPECK:

First let me emphasize that if there is any criticism of the attempts to solve this problem that have been made by people working on the organic aspects of the browning reaction, that is, the organic chemistry of it, it should be remembered that all of us have kept in mind all along the more practical problems. Perhaps I am not justified in saying that there has been any actual criticism of this approach.

In our own laboratory, our results indicate that the amino acids are very important in this kind of browning, and we feel that the information we are accumulating may be applied to actual products research. We have in mind, for example, applications useful to the citrus industry. In citrus products you cannot get rid of the reducing sugars without losing the most essential part of the product. It might be possible, however, to get rid of some free amino compounds which are possibly catalyzing the degradation of the reducing sugars. We have in mind, in fact, a program whereby we might free citrus juices from any traces of amino acids. But if we were to attempt to do this on our present budget with our present manpower, we would be spreading ourselves extremely thin. We would probably get nothing done.

We have a knotty problem when it comes to manpower. Our present resources seem to be very much limited. We do not expect to get *any* graduate students to speak of in the future—at least, not within the next couple of years. Therefore, I can scarcely overemphasize the fact that it is not a matter of our keeping graduate students in graduate school by means of contract research; it is virtually a case of getting any graduate students at all. As to getting graduate students interested in doing anything on a problem like this—assuming a few are available—you would have to get down on your knees to persuade them.

We could probably keep five people busy—if somebody would give us the manpower—working on practical problems. At the present time, we do not have the money and we do not have the manpower and we do not foresee any manpower pool ahead to make it easy to recruit scientific skill for work of this nature in the future.

MITCHELL:

At this point I should like to ask Dr. Hunter to give us his reactions.

HUNTER:

One purpose of the meeting today was to consider the browning program in relation to the money spent on this problem in the past few years—some $180,000. It does nobody any harm to sit down once in a while and search his soul. This meeting today was called for the purpose of doing just that. Are we on the right track?

We kept this meeting small, deliberately. We called in only people who are in the middle of it—both the people who are working under contract on the problem today and those who have worked on it in the past. Even though at the moment you may not have concrete suggestions or recommendations as to where we should go, when you have had an opportunity, think over what you have heard today and what you have been thinking about yourself. Let us have your concrete recommendations as to problems which should be attacked. They will be most useful in budgetary and program planning in the months ahead.

Should we terminate some of the investigations we are undertaking today? Should we continue them? Are there avenues that in your judgment are more profitable than the ones we are presently following? After all, we are spending your money, and there is an increasing remonstrance against high taxes. We want to spend where, in your best judgment, we will get the most out of your dollar. In general, that is the purpose of having you here. I am glad you came; we appreciate your interest and your continued interest.

IV. Review of Browning Reaction Studies In Relation to Product Applications

by

Harold S. Olcott, Western Regional Research Laboratory,[1]
Albany, California

The Quartermaster Corps Subsistence Research and Development Laboratory and its expert consultants recognized early in World War II that there was a lack of sufficient fundamental information either to account for the deteriorative changes that occur in dehydrated foods or to help in changing the specifications in order to minimize them. A conference was held in Chicago in June, 1944, to discuss the biochemistry of such changes. In his outline of the objectives, Dr. George F. Stewart discussed the three main causes of deterioration, namely: (1) the amino-aldehyde or Maillard reaction, (2) oxidative reactions, and (3) enzymatic changes. He emphasized the desirability of further research in these fields and the possibilities of progress resulting from the cross-fertilization of ideas. Emphasis on the amino-aldehyde reaction was justified; dehydrated foods, particularly eggs and vegetables, were being found inedible at the point of consumption. The products were discolored, difficult to rehydrate and had developed off-flavors and odors. These symptoms were recognized as being typical of a Maillard-type reaction. Despite a growing awareness of the importance of this reaction in several technological fields, no concerted effort had been made to bring together all interested workers, or to emphasize to other research personnel the problems that required solution.

Conference No. 1 and the subsequent effort by the Quartermaster Laboratory to encourage, support and coordinate a nationwide program of study on the "browning" reaction were eminently successful. Improvements in specifications and products followed. During the post-war period this support was wisely continued with the result that information of immediate utility was available when it again became necessary to consider large-scale procurements of dehydrated and concentrated foods. The food industries also owe a debt to the Quartermaster Laboratory for bringing to their attention both the background information and the need for research on browning. Processed food technology, apart from military procurement, has profited from the stimulus.

Future Program. Despite progress in knowledge of the reactions that occur during "browning" and also in the development of methods for their control, it will be obvious, from this brief review and the papers which have preceded, that continued effort is required. The complexity of the reactions suggests that research may be time-consuming and costly. Immediate practical results should not be expected. But the problem has such long-term importance that continued encouragement and financial support are essential.

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[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

The Amino-Aldehyde, Maillard, "Browning," or Melanoidin Reaction. Many able reviews of the literature on "browning" were prepared under Army auspices. One, in revised form, has recently been published (J. P. Danehy and W. W. Pigman, Reactions Between Sugars and Nitrogenous Compounds, in *Advances in Food Research,* Vol. III, Academic Press, Inc., New York, pp. 241-290). It therefore does not appear necessary to attempt a detailed review here. However, a simplified undocumented resumé of what is known about the amino-aldehyde reaction may help the reader to orient himself in the research program presently being conducted for the Qm. Corps. The several other types of reactions which are possibly involved in the discolorations of dehydrated foods will not be discussed.

Maillard noted the following sequence of reactions in concentrated solutions containing mixtures of an amino acid (such as glycine, alanine, glutamic acid, etc.) and a reducing sugar (glucose, lactose, etc.). The solutions first turn yellow, then red, then brown. As the reactions proceed there is development of caramel-like flavors and odors, evolution of carbon dioxide, and eventually separation of insoluble dark brown humin-like material. The changes that occur in dried foods containing some moisture, such as dehydrated eggs, milk, and potatoes, have been shown to be of similar nature. In the hundreds of papers which have appeared since Maillard's, numerous contributory factors have been described. The following outline of necessity neglects a detailed discussion.

Nature of the Aldehyde. Investigators have spent most of their efforts on the reducing sugars, since these are the "bad actors" in nature. Pentoses react more rapidly than hexoses, which, in turn are more active than di- or trisaccharides. The non-reducing sugars brown only under conditions which could involve hydrolysis. The reactions of fructose are not entirely understood. The browning reaction is also readily demonstrable with many simpler aldehydes with the exception of formaldehyde. Acetaldehyde browns 35 times as fast as an equivalent concentration of glucose with bovine serum albumin. Glycolaldehyde is also reactive. Enders considered methylglyoxal to be an important intermediate carbohydrate fragment in the browning reaction. Furfural and hydroxymethylfurfural have also been proved to be intermediate reaction products. Whether sugars are directly involved or whether they react only after decomposition seems to depend upon the conditions under which the reactions are run.

Nature of the Amino Compound. All aliphatic compounds containing primary or secondary amino groups react but at greatly differing rates, depending upon the structure of the amine. Simple amines brown rapidly. The sterically hindered alpha-aminoisobutyric acid reacts very slowly compared to other amino acids. Serine reacts more slowly than alanine, which in turn reacts more slowly than beta-alanine. The amino groups of proteins and phospholipids are available for the reaction. Blocking of the amino groups of proteins very greatly inhibits rate of browning.

Nature of the Reaction. A simple formulation of the reaction of an aldehyde with an amine is shown in Figure 1. The first and immediate reaction in solution is a reversible combination of amine and aldehyde. Evidence

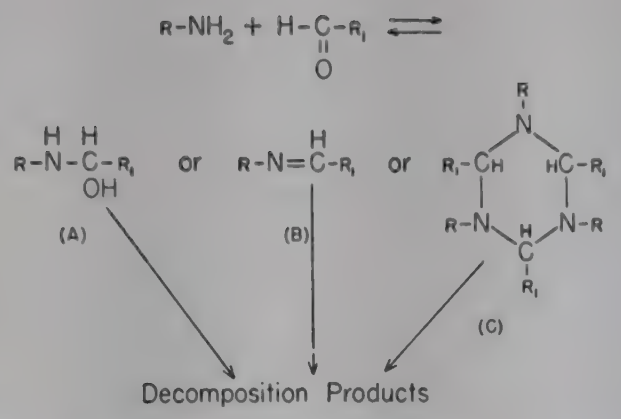


FIG. 1. Reaction of an aldehyde with an amine.

favors formulation (A) rather than (B) for reactions with sugars; (C) may be the structure for the first reaction products of low molecular weight amines and aldehydes. These colorless intermediates are hydrolyzable with recovery of the amine and aldehyde. Evidence of the reaction is the drop in pH which reflects the lower basicity of the substituted amine. The second, irreversible series of steps has been the subject of numerous investigations, some of which have been outlined in the preceding pages. These subsequent reactions are characterized by disappearance of amino nitrogen, formation of colored products, appearance of reducing activity and the evolution of carbon dioxide: in other words, the Maillard syndrome occurs.

Effect of pH. Within the pH range 5 to 8 the browning reaction is markedly accelerated with increasing alkalinity. Observations have been made in the more acid regions which suggest that the browning reactions may differ qualitatively from those in the neutral region. Definite evidence has been obtained that organic acids may play an important role in the browning of such products as dried apricots, in which the acid concentration corresponds to pH 3-4.

Effect of Concentration. The rates of the amino-aldehyde reaction are extremely sensitive to the concentration of the reactants, becoming much more rapid as the concentration increases. This, of course, accounts for its practical importance in concentrated and dehydrated foods. In the solid state the reactions proceed most rapidly at moisture levels between 10 and 20%—enough apparently to permit interreaction of the components. More tends to dilute the components; less, to prevent the combination. However, once the primary condensation occurs, water may not be necessary for the subsequent breakdown.

Effect of Temperature. The browning reaction is also extremely sensitive to heat. Carefully measured reaction rates, both in solution and in the solid state, have shown that the reaction is speeded up to the extent of 3 to 7 times for each 10°C. rise in temperature. There is evidence that the nature of the reaction does not change between room temperature and 65°; possibly different kinds of reactions begin to become important at higher temperatures.

Effect of Atmosphere. Most investigators have found that browning reactions are not directly affected by the atmosphere with which the systems

are in contact. Nearly identical rates of browning have been observed both with dried vegetables and model systems held in air or nitrogen. On the other hand, dried eggs brown less rapidly in air than in nitrogen, and rate of liberation of carbon dioxide increases.

Practical Aspects. It is obvious that there are two conditions where the "browning" reactions can become particularly serious in food handling: first, in the process of removing water from the material to be dehydrated and, second, during subsequent storage. As the foodstuff is being dried, the moisture content passes through the region in which the rate of browning is maximum, usually at elevated temperature. Thus caramelization, browning, and off-flavor and odor development are often encountered in improperly dried products. The same reactions occur but more slowly if the dried products are held in storage under conditions which favor the reactions, namely, relatively high temperatures or moisture contents.

Methods of Control. Methods for inhibiting the browning reaction follow from the foregoing brief discussion:

1. *Removal of part or all of the aldehyde or amino components or both.* This has been achieved in some commodities. For example, a simple kind of control can be exercised by using raw material with low reducing sugar or amino contents. Thus potatoes should not be dehydrated if their content of glucose is greater than 2%. With eggs, removal of the glucose by fermentative or enzymatic means greatly increases the storage life of the dehydrated product. Similarly, extraction of the soluble "browning" constituents of solid commodities permits preparation of more stable dehydrated products.

2. *Decreased moisture content.* The problem of arriving at low moisture levels is complicated by the fact that, as products dry, the removal of the residual water becomes more and more difficult, and the times and temperatures required are costly and may damage the product. The use of a desiccant in the sealed package looks promising inasmuch as the moisture can thus be removed from the product during normal storage.

3. *Acidification.* The addition of acid to eggs achieves stabilization insofar as the rate of "browning" of the dried eggs is decreased. With "piece" products, the problem of penetration and the adverse effect of acid on, for example, chlorophyll, are practical detriments.

4. *Temperature control.* Dehydration schedules for piece foods must be governed by the need to achieve the highest efficiency possible with least damage to the product. This means that higher air temperatures can be used at the beginning of the run when the material is wet and evaporation is rapid, and lower temperatures toward the end when the rate of drying is limited by the rate of diffusion of water from the center of the piece to the surface. The removal of water during the last stages in bins rather than in the dehydrators is advantageous since it permits handling of large amounts of material. Low-temperature storage of the final dehydrated product would obviously be beneficial but in practice may be difficult.

5. *Additives.* The use of "anti-browning" additives has been investigated in some detail. Sulfites are the most effective but sulfhydryl compounds, formaldehyde and a few others have also been found to decrease the rate of browning. As a result of its proven effectiveness, low levels of sulfite are permitted or required in several dehydrated products. If added prior to dehydration, sulfites have the additional advantage of protecting the commodity during its exposure to high temperature. The ability of sulfite to inhibit browning during storage is adversely affected by such factors as high moisture content or the presence of air, both of which accelerate its rate of disappearance from dried foods.

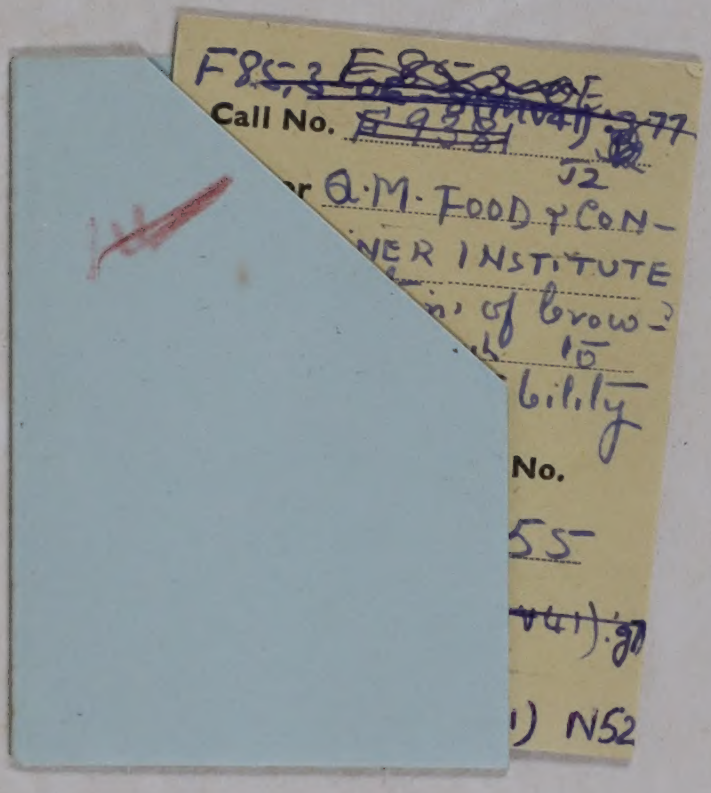
Beneficial aspects. End-products of the amino-aldehyde reaction are often desirable rather than to be avoided. The color and flavor of bread crust, the outer portion of roasted meats, toasted breakfast foods, maple sirup, molasses, etc., are in all likelihood the result of browning reactions. Experimentally it has been shown that products of the reactions of amino acids with sugars often have desirable odors, resembling maple, bread, beer, and others. In part these are due to the presence in the reaction product of mixture of aldehydes derived from the amino acids but containing one carbon less.

Summary. The results of the many observations and experiments since the time of Maillard have led to the various solutions to browning deterioration which have been touched upon in the preceding paragraphs. The papers which precede have added more recent and more detailed information. Although substantial gains have been made, it is to be expected that continued study will result in still further advances in our ability to understand and control the browning reaction.

Addendum. A résumé of Western Regional Research Laboratory work bearing on the "browning" reaction was requested for inclusion in this symposium. In place of a detailed summary, the interested reader is invited to consult the following list of papers. Most of these investigations were undertaken at the behest of the Quartermaster Food and Container Institute.

1. Changes in Stored Dried Eggs. Source of Fluorescence. H. S. Olcott and H. J. Dutton. *Ind. Eng. Chem.*, **37**, 1119-1121 (1945).
2. Changes in Stored Dried Eggs. Role of Phospholipids and Aldehydes in Discoloration. B. G. Edwards and H. J. Dutton. *Ind. Eng. Chem.*, **37**, 1121-1122 (1945).
3. Changes in Stored Dried Eggs. Spectrophotometric and Fluorometric Measurement of Changes in Lipids. H. J. Dutton and B. G. Edwards. *Ind. Eng. Chem.*, **37**, 1123-1126 (1945).
4. Determination of Carotenoids and Lipid Amine-Aldehyde Products in Dehydrated Egg. H. J. Dutton and B. G. Edwards. *Ind. Eng. Chem.*, Anal. Ed. **18**, 38-41 (1946).
5. Changes in Color of Dehydrated Eggs during Storage. H. J. Dutton and B. G. Edwards. *Ind. Eng. Chem.*, **38**, 347-350 (1946).
6. Relative Stabilities of D-Glucose Amino Derivatives. A. Mohammad and H. S. Olcott. *J. Am. Chem. Soc.*, **69**, 969 (1947).
7. Dehydrated Egg Powders. Factors in Palatability of Stored Powders. M. M. Boggs and H. L. Fevold, *Ind. Eng. Chem.*, **38**, 1075-1079 (1946).

8. Browning of Dehydrated Vegetables during Storage. R. R. Legault, W. F. Talburt, A. M. Mylne, and L. A. Bryan. *Ind. Eng. Chem.*, 39, 1294-1297 (1947).
9. The "Browning" Reaction of Proteins with Glucose. A. Mohammad, H. Fraenkel-Conrat, and H. S. Olcott. *Arch. Biochem.*, 24, 157-179 (1949).
10. The Reactions of Proteins with Acetaldehyde. A. Mohammad, H. S. Olcott, and H. Fraenkel-Conrat. *Arch. Biochem.*, 24, 270-280 (1949).
11. Measurement of Non-Enzymatic Browning of Dehydrated Vegetables during Storage. C. E. Hendel, G. F. Bailey, and D. H. Taylor, *Food Technol.*, 4, 344-347 (1950).
12. Role of Glucose in the Storage Deterioration of Whole Egg Powder. I. Removal of Glucose from Whole Egg Melange by Yeast Fermentation before Drying. L. Kline and T. T. Sonoda. *Food Technol.*, 5, 90-94 (1951).
13. Role of Glucose in the Storage Deterioration of Whole Egg Powder. II. A Browning Reaction Involving Glucose and Cephalin in Whole Dried Eggs. L. Kline, J. E. Gegg, and T. T. Sonoda. *Food Technol.*, 5, 181-187 (1951).
14. Role of Glucose in the Storage Deterioration of Whole Egg Powder. III. Effect of Glucose Removal before Drying on Organoleptic, Baking and Chemical Changes. L. Kline, H. L. Hanson, T. T. Sonoda, J. E. Gegg, R. E. Feeney, and H. Lineweaver. *Food Technol.*, 5, 323-331 (1951) .
15. Browning of Dehydrated Sulfited Vegetables during Storage. R. R. Legault, C. E. Hendel, W. F. Talburt, M. F. Pool. *Food Technol.*, 5, 417-423 (1951).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